PARENTAL AGE EFFECTS AND THE EVOLUTION OF SENESCENCE IN *LEMNA MINOR*

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A Thesis
Submitted to the School of Graduate Studies
of the University of Lethbridge
in Partial Fulfillment of the
Requirements for the Degree

DOCTOR OF PHILOSOPHY

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Date of Defence: April 17, 2015

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ABSTRACT

Senescence is characterized by age-related deterioration within individual organisms and a resultant decline in rates of survival or reproduction. Such declines seem inherently maladaptive, but occur nonetheless in a wide range of species. My thesis contributes to the questions of (i) why senescence is common in nature, and (ii) why patterns of senescence sometimes vary markedly both within and among species. With respect to why senescence is common, most evolutionary theory on senescence makes the simplifying assumption that all offspring are of equal quality. I show that this assumption does not hold in the aquatic plant *Lemna minor*, and develop a theoretical model to investigate how age-related declines in offspring quality influence the 'force' of natural selection. With respect to variation in patterns of senescence, I describe a common garden experiment demonstrating a high degree of among-population consistency in life expectancy and rates of senescence in *L. minor*.

ACKNOWLEDGEMENTS

I would like to thank Rob for being an outstanding advisor, Sarah Bogart for assistance in the field and lab, and my labmates and fellow graduate students for inspiration and for all the great times. Research funding was provided by the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Alberta Conservation Association's ACA Grants in Biodiversity Program. I gratefully acknowledge graduate funding provided by NSERC through the Alexander Graham Bell Canada Graduate Scholarships Program, and the Government of Alberta through the Queen Elizabeth II Graduate Scholarships Program.

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CHAPTER 1: GENERAL INTRODUCTION

It is indeed remarkable that after a seemingly miraculous feat of morphogenesis a complex [animal] should be unable to perform the much simpler task of merely maintaining what is already formed. (George C. Williams, 1957)

Demographic senescence is characterized by a progressive decline in rates of survival or fecundity with increasing age (reviewed in Partridge and Barton 1993, Kirkwood and Austad 2000, Hughes and Reynolds 2005, Williams et al. 2006, Sherratt and Wilkinson 2009). Such declines generally coincide with various forms of physiological deterioration or damage. For example, demographic senescence (hereafter simply 'senescence') has been associated with the accumulation of harmful protein aggregates in bacteria (Lindner et al. 2008) and oxidized proteins in yeast (Aguilaniu et al. 2003), the attrition of telomeres in eukaryotes (Monaghan 2010), declining immune function in various animals (Shanley et al. 2009), reduced rates of plant photosynthesis (Munné-Bosch 2007), and the accumulation of mitochondrial DNA mutations in ageing mammals (Wallace 1999). Clearly these physiological changes are deleterious to the ageing individual, so why do they occur? Why does natural selection not work to perpetually delay bodily deterioration and maximize the amount of time that an organism has to reproduce?

EVOLUTIONARY THEORY ON SENESCENCE

Modern theories for the evolution of senescence were born out of the realization that, because survivorship to a given age must be a monotonically

declining function of age (assuming any level of mortality greater than zero), the force of natural selection acting on age-specific vital rates will also tend to decline with increasing age (Medawar 1952, Williams 1957, Hamilton 1966). Intuitively, if only a tiny fraction of a given population reaches a particular age class, there is relatively little value in a mutation that increases vital rates at or beyond that age class (at least compared to a mutation similarly affecting an earlier age class that a greater proportion of the population will attain). This idea – that the force of selection should decline with age – led to three prominent theories for the evolution of senescence: mutation accumulation (Medawar 1946, 1952), antagonistic pleiotropy (Williams 1957), and disposable soma (Kirkwood and Holliday 1979, Kirkwood and Rose 1991).

The mutation accumulation theory proposes that senescence occurs because mutations with late-acting detrimental effects go relatively unopposed by natural selection, and therefore tend to accumulate in the gene pool (Medawar 1946, 1952). For example, the onset of Huntington's disease in humans often occurs around middle age (Warby et al. 1998), by which time the force of natural selection is relatively weak. Even prior to Medawar's formalization of the mutation accumulation theory, geneticist and evolutionary biologist J. B. S. Haldane recognized that early humans would have reproduced and died long before the onset of Huntington's disease, which may have prevented the allele causing Huntington's from being efficiently opposed by selection (Haldane 1941).

A second theory for the evolution of senescence, the antagonistic pleiotropy theory, proposes that senescence is caused by the accumulation of certain

pleiotropic genes – genes that are beneficial at a young age but detrimental at older ages (Williams 1957). If the force of natural selection declines with age, then there is a selective premium on early age classes, and antagonistically pleiotropic mutations may sometimes be favoured even if the harm done at later ages exceeds the benefit to earlier age classes. But how could a gene be beneficial at one age and detrimental at another? The most recent of the three major theories for the evolution of senescence, disposable soma, offers an explanation for antagonistic pleiotropy. Disposable soma theory posits that senescence is the result of an optimal allocation of resources between two competing activities: reproduction and somatic repair (Kirkwood and Holliday 1979, Kirkwood and Rose 1991). Because there are multiple potential uses for a finite pool of resources (e.g. growth, reproduction, somatic repair), investment in any one activity involves an opportunity cost such that the invested resources cannot be allocated to any other activity. Any allele that has the effect of increasing an individual's relative allocation of resources to reproduction could therefore be antagonistically pleiotropic because expression of that allele would indirectly decrease the allocation of resources to somatic repair (which may adversely affect the individual at a later age). Although there are few clear examples of genes with antagonistically pleiotropic, age-specific effects on fitness (Leroi et al. 2005), there is both experimental and observational evidence suggesting that investment in early reproduction comes at a cost to later reproduction and survival (Rose 1984, Westendorp and Kirkwood 1998, Charmantier et al. 2006, Nussey et al. 2006, Penn and Smith 2007). Such a trade-off is consistent with the antagonistic pleiotropy and disposable soma theories for the evolution of senescence.

The theories of senescence described above emphasize the failure of natural selection to oppose genes with late-acting detrimental effects. An alternative consequence of declining selection that has only recently received attention is the failure of natural selection to favour genes with beneficial effects. For example, given that the proximate causes of senescence seem to involve physiological deterioration due to environmental damage, why does natural selection not produce organisms that are invulnerable to environmental damage? The disposable soma theory suggests that invulnerability to damage requires a non-optimal allocation of resources to somatic repair (at the expense of reproduction). Although this explanation is intuitive, it remains somewhat unsatisfactory because the disposable soma theory relies on the perhaps unwarranted assumption that somatic repair mechanisms have already evolved and are themselves invulnerable to damage (Laird and Sherratt 2010). A more recent theory for the evolution of senescence, the reliability theory of senescence, does not assume pre-existing, invulnerable, costly repair mechanisms, yet can still account for the evolution of senescence due to the failure of natural selection to favour genes with beneficial effects (Laird and Sherratt 2009, 2010). The reliability theory of senescence envisions mortality as resulting from the failure of vital genes or gene products due to random environmental damage. One way to reduce vulnerability to environmental damage is to evolve redundant copies of vital genes which act as backups in the event that the original gene or gene product is damaged to the point of failure. Models based on reliability theory show that this type of redundancy is favoured by natural selection, but only up to a point. Even if carrying redundant gene copies is not inherently costly, natural

selection will not lead to invulnerability, but rather will produce only as much redundancy as will be beneficial within the *typical* lifespan of individuals in a population. Because any finite and nonzero level of redundancy will give rise to senescence (under certain assumptions), the reliability theory of senescence suggests that senescence can evolve in an originally non-senescing population as a byproduct of selection for increased – though not infinite – redundancy (Laird and Sherratt 2009, 2010).

PARENTAL AGE EFFECTS AND THE EVOLUTION OF SENESCENCE

Most theory on the evolution of senescence makes the implicit, simplifying assumption that all offspring are of equal quality, so that fitness and the force of selection depend only on age-trajectories of survival and fecundity (e.g. Hamilton 1966, Abrams 1993, Pedersen 1995, Sozou and Seymour 2004, Baudisch 2005). In nature, this assumption does not always hold. Across a wide range of taxa, offspring tend to decline in quality with increasing parental age (referred to generally as a 'parental age effect'). For example, declines in offspring lifespan with increasing parental age have been observed in rotifers (Lansing 1947, 1948), fruit flies (Priest et al. 2002), ladybird beetles (Singh and Omkar 2009), duckweeds (Ashby and Wangermann 1949), and humans (Bell 1918, Gavrilov and Gavrilova 1997). Other offspring traits may also be affected by parental age, including size (Ashby et al. 1949), development time (Berkeley et al. 2004, Benton et al. 2008), diapause frequency (reviewed in Mousseau and Dingle 1991), and age-trajectories of survival (Descamps et al. 2008) and fecundity (Bouwhuis et al. 2010, Gillespie et al. 2013a).

Why do parental age effects matter? As others have noted (e.g. Kern et al. 2001, Priest et al. 2002), age-related declines in offspring quality are conceptually similar to demographic senescence – classically defined as a decline in the rate of survival or fecundity with increasing age. That is, all else being equal, a lineage not subject to age-related declines in offspring quality should have greater future representation than one that is. Furthermore, recent theoretical results suggest that age-related declines in offspring quality may increase the rate of age-related decline in the force of natural selection (Pavard et al. 2007a,b, Pavard and Branger 2012, Gillespie et al. 2013a), which could pave the way for the evolution of more rapid intrinsic decline (i.e. increased rates of senescence). To understand this intuitively, recall my previous argument that there is relatively little value in a mutation that increases vital rates for an advanced age class that few individuals attain. The relative value of this hypothetical 'late-acting' mutation (relative to an identical mutation that affects an earlier age class) would be further reduced if individuals in the affected age class inevitably produced offspring of relatively low quality (again, relative to the earlier age class). Thus, parental age effects should generally reduce the force of selection on late age classes. Apart from the above-cited works (i.e. Pavard et al. 2007a,b, Pavard and Branger 2012, Gillespie et al. 2013a), parental age effects have been largely omitted from evolutionary theory on senescence. Given that parental age effects are common in nature, and potentially influence age-trajectories of selection, a greater incorporation of parental age effects into evolutionary theory on senescence is warranted. This will be the first of my two main thesis objectives, as further discussed below.

DIVERSITY IN PATTERNS OF SENESCENCE

Ideally, evolutionary theory on senescence should explain not just why senescence might evolve, but also help to explain the remarkable diversity in rates and patterns of senescence seen in nature (e.g. Jones et al. 2014). For instance, among vertebrate animals, maximum lifespan varies from just a few months in the Labord's chameleon (*Furcifer labordi*) to over 200 years in the rougheye rockfish (*Sebastes aleutianus*) (de Magalhães and Costa 2009). An even wider range of variation occurs among vascular plants, with life expectancies ranging from a few months to thousands of years (Noodén 1988). Life expectancy is not necessarily a good proxy for the rate of senescence (Baudisch 2011), but alternative metrics that relate more closely to the rate of senescence suggest similar variability, both within and among species (Silvertown et al. 2001, Reznick et al. 2004, Baudisch 2011, Baudisch et al. 2013, Jones et al. 2014). What extrinsic forces underlie this variation?

A great deal of research examining variation in rates of senescence has focused on a single prediction (attributed to Williams 1957) – that populations subject to relative high extrinsic mortality rates (e.g. age-independent mortality due to predation, disease, environmental insult, etc.) should evolve relatively rapid rates of intrinsic decline (i.e. a relatively rapid rate of senescence). This widely-tested prediction (reviewed in Williams et al. 2006) has received mixed support based on among-species comparative studies (Silvertown et al. 2001, Ricklefs 2010), within-species or -genera common garden experiments (Dudycha 2001, Reznick et al. 2004, Terzibasi Tozzini et al. 2013), and experimental evolution in laboratory

environments (Stearns et al. 2000, Ackermann et al. 2007). One possible explanation for the inconclusive results is that the above-described prediction does not actually follow from any formal theory (Abrams 1993, Caswell 2007). Although the verbal arguments put forth by Williams (1957) and others are intuitive, a change in age-independent mortality rates is not expected to alter the age-trajectory of selection based on formal mathematical theory (Abrams 1993, Caswell 2007). Given that Williams' prediction has arguably been the most widely-tested prediction relating (or perhaps not relating) to evolutionary theory on senescence, a great deal of the existing variation in rates of senescence remains unexplained. This unexplained variation is the subject of my second main thesis objective, as further discussed below.

SENESCENCE IN PLANTS

A number of authors have suggested that the occurrence of senescence among vascular plants should be relatively rare (Vaupel et al. 2004, Peñueles and Munné-Bosch 2010) or even that plants are predisposed to 'immortality' (Silvertown et al. 2001). Such views are due to certain unique aspects of the plant form and life history. For example, unlike other organisms that exhibit determinate growth, many vascular plants exhibit indeterminate (i.e. continual) growth and regeneration via totipotent apical meristems. This indeterminate growth pattern potentially allows for a continual increase in reproductive potential with age, which may limit or even reverse the expected age-related decline in the force of natural selection (Vaupel et al. 2004). Indeed, analyses of published population projection

matrices suggest that many perennial angiosperms are not subject to senescence (Silvertown et al. 2001, Baudisch et al. 2013). That said, there are certainly some clear cases of senescence among perennial plants (e.g. Roach et al. 2009, Ally et al. 2010, Pujol et al. 2014), and of course, many semelparous plant species that exhibit rapid senescence following a single reproductive event.

Some authors have suggested that, compared to animals, there has been relatively little investigation into demographic senescence in plants (e.g. Watkinson 1992, Thomas 2003, Monaghan et al. 2008, Salguero-Gómez et al. 2013). Presumably, this imbalance stems partly from the influence of gerontology on senescence research (with its emphasis on human ageing), but may also relate to characteristics of plants that tend to make the study of demographic senescence in plants more difficult than in animals. Perhaps the most obvious of these intractable characteristics is the extreme longevity of certain plants (Noodén 1998), which can make it prohibitively time-consuming to track samples of individuals from birth to death. A second difficulty in the study of demographic senescence in plants comes from the fact that many plants reproduce both clonally and sexually, which can make it difficult to determine the unit that natural selection acts on (Buss 1983, Tuomi and Vuorisalo 1989). Selection may occur at the level of modules produced via clonal reproduction (ramets), at the level of groups of ramets derived from the same zygote (genets), or at both levels simultaneously. Likewise, senescence could theoretically occur in ramets only, genets only, or both ramets and genets (Pedersen 1995, Gardner and Mangel 1997). Despite the difficulties inherent in studying demographic senescence in plants, the incredible diversity of life histories and

patterns of senescence found among plants makes this group a potentially fruitful target for research into the evolutionary foundations of senescence.

OBJECTIVES AND THESIS OVERVIEW

My thesis has two primary objectives. The first (Chapters 2-4) is to characterize parental age effects in my species of interest (the small aquatic plant *Lemna minor*; described in the next section), and examine how such effects might modify the force of natural selection on age-specific vital rates. The second objective (Chapter 5) is to examine among-population (intraspecific) variability in rates of senescence in *L. minor*, and test for environmental correlates thereof.

Objective 1: Parental age effects and the force of natural selection

As noted above, parental age effects have been largely omitted from evolutionary theory on senescence, despite the conceptual similarly between age-related declines in offspring quality and age-related declines in the two 'classic' fitness components – survival and fecundity. Recent theoretical results suggest that parental age effects, where they occur, may lead to a steeper decline in the force of natural selection with age (Pavard et al. 2007a,b, Pavard and Branger 2012, Gillespie et al. 2013a), which could pave the way for the evolution of more rapid rates of intrinsic decline (i.e. increased rates of senescence).

My first objective is to characterize parental age effects in my study species, *L. minor*, and develop a population projection model that can be used to examine whether such effects modify the force of natural selection on age-specific vital rates.

Early studies on senescence in *L. minor* (described in greater detail in the next section) documented a decline in offspring size, lifespan, and total reproductive output with increasing parental age (Wangermann and Ashby 1950, 1951). Because there is a premium on early reproduction, lifespan and total reproductive output may be poor measures of overall fitness (Partridge and Barton 1996). Therefore, in Chapter 2, I follow up on those early studies to specifically examine whether offspring *fitness* declines with increasing parental age in *L. minor*, using a composite fitness metric (the intrinsic rate of increase) that takes into account not just lifespan and total reproductive output, but also the timing of reproduction. Also in Chapter 2, I ask whether *L. minor* is subject to age-related declines in the two classic fitness components – survival and fecundity. In brief, I found that survival, fecundity, and offspring fitness all declined with increasing age. A modified version of Chapter 2 is published in the journal Function Ecology (see Appendix 1 for more information).

Next, in Chapter 3, I examine whether age-related declines in offspring fitness carry over across multiple generations (e.g. is fitness affected by grandparental age, great-grandparental age, etc.?). Intuitively, all else being equal, a parental age effect that extends across multiple generations should have a greater impact on fitness than one that 'resets' at each new generation. My results suggest that parental age effects on offspring size do extend across multiple generations in *L. minor*, but parental age effects on offspring fitness (i.e. the intrinsic rate of increase) do not.

In Chapter 4, I develop a population projection model that incorporates parental age effects, and use this model to examine how the parental age effect I observed in *L. minor* (Chapters 2 and 3) might modify the force of natural selection

on age-specific vital rates. In accordance with results from recent theory, I find that the parental age effect in *L. minor* tends to increase the force of selection on early age classes and reduce the force of selection on late age classes, compared to what is expected in the absence of the parental age effect.

Objective 2: Among-population variation in senescence

The second objective of my thesis is to examine whether there is genetically-based, among-population variability in rates of senescence in *L. minor* (focusing on actuarial senescence; i.e. age-related declines in survival), and whether such variation, if it exists, correlates with variation in environmental characteristics at the sites of population origin. Among plants and animals, there is extensive variation in life expectancy and rates of senescence, both within and among species. However, relatively little is known about the evolutionary pressures that underlie such variation. Relevant research to date has largely focused on a single prediction – that increased extrinsic mortality should favour the evolution of more rapid intrinsic decline. But support for this prediction is mixed (Williams et al. 2006), and it turns out that the prediction does not really follow from evolutionary theory on senescence anyway (Abrams 1993, Caswell 2007).

As pertains to the objective described above, in Chapter 5, I describe a common garden experiment examining genetically-based variation in rates of actuarial senescence among 28 putative strains of *L. minor*, initially collected from 23 wetlands in Alberta. Here, I am interested both in the degree of variability among strains, and whether such variation might relate to environmental characteristics of

the sites of origin. I focus on environmental characteristics that are known to influence life history traits of *L. minor* on an ecological time scale (i.e. via phenotypic plasticity), including temperature, nutrient concentrations, and water salinity. In brief, I found little among-strain variation in life expectancy or rates of actuarial senescence, but greater variation in other life history traits including frond size and total reproductive output. The only relationship between life history traits in the common garden and environmental characteristics at the sites of origin was a relatively weak negative relationship between frond size and nutrient concentrations (nitrogen and phosphorus).

STUDY SPECIES

Lemna minor L. is a tiny aquatic plant belonging to Lemnoideae (the duckweeds), a subfamily said to comprise "the simplest and smallest of flowering plants" (Hillman 1961, p. 222). It floats on the surface of slow-moving fresh water bodies, occurring on every continent except Antarctica (Landolt 1986, p. 275-282). Reproduction in *L. minor* is predominantly asexual via budding (exclusively so under laboratory conditions), though flowering does occasionally occur in the wild (Landolt 1986, p. 167-169). Proliferation can be extremely rapid, and duckweed will often form dense mats when conditions are favourable (Fig. 1-1). Duckweed species, in fact, have some of the highest documented relative growth rates among vascular plants (Ziegler et al. 2015).

Individual duckweed plants (i.e. ramets; Fig. 1-2) have a reduced shoot architecture consisting of a flattened, leaf-life structure (usually called a 'frond'),

with a single root emanating from the lower surface. The term frond is used to describe the main body unit because it is thought to derive from a combination of leaf and stem tissues (Lemon and Posluszny 2000). Though not the smallest among the duckweeds (that honour belongs to the genus *Wolffia*), fronds of *L. minor* are very small, with a surface area of about 2-9 mm^2.

With respect to asexual reproduction, offspring (often referred to as daughters) develop in alternating succession from one of two meristematic pockets located laterally on either side of the parent frond (Lemon and Posluszny 2000). Within clonal strains (i.e. genets), there is a high degree of consistency with respect to the pocket (right or left) from which the first daughter develops. The first daughter to develop from a given parent is initiated very early – while the parent is still developing within its own parent (Lemon and Posluszny 2000). As daughter fronds develop, they remain joined to their parent via a structure called the 'stipe', which eventually severs once the daughter is mature (Landolt 1986, p. 66-67). Because first daughters initiate so early, and because the stipe occasionally remains intact for a prolonged period of time, many generations of fronds may be joined together growing in an interconnected cluster.

A number of studies have investigated aspects of ramet senescence in the genus *Lemna* (Ashby and Wangermann 1949, Ashby et al. 1949, Wangermann and Ashby 1950, Ashby and Wangermann 1951, Wangermann and Ashby 1951, Wangermann 1952, Ashby and Wangermann 1954, Wangermann and Lacey 1955, Claus 1972). Some results from these early studies are pertinent to my own research, but others are difficult to interpret from the perspective of modern

research on senescence. For instance, the authors cited above were not generally interested in age-trajectories of survival and fecundity, though some of their results relate nonetheless. Because inferential tests were rarely performed, sample sizes not always reported, and methodology not always sound by modern standards, the evidence is sometimes difficult to assess. My own interpretation of the pertinent results from these studies is as follows: (**1**) fair evidence that *L. minor* exhibits age-related declines in survival; (**2**) mixed evidence for age-related declines in fecundity; (**3**) strong evidence for parental-age-related declines in offspring size, lifespan, and total reproductive output; (**4**) strong evidence for plasticity in lifespan with respect to environmental characteristics (temperature, day length, and nitrogen availability, but not light intensity); and (**5**) fair evidence for among-strain variability in lifespan.

With respect to (**1**), Ashby et al. (1949) suggest that the lifespan of *L. minor* is 'fixed' (i.e. exhibits low variance), which, in retrospect, is indicative of an increase in mortality rate (= decline in survival rate) with increasing ramet age. I say this because, if mortality is constant with age (age-independent mortality rate = μ), then the probability of dying at any given age is given by the exponential function, which has a mean of μ^{-1} and variance of μ^{-2} (variance > mean if $\mu < 1$). Because they did not report variance or sample size, I cannot definitively say whether Ashby et al. observed variances in lifespan smaller than the means (which would indicate a decline in survival rate with increasing ramet age), but based on their reported standard errors, it is very likely that they did (i.e. their sample sizes would have had to be very large for the reported standard errors to reflect variance ≥ mean; e.g. N > 1000).

As with survival rates, the above-cited authors were not specifically concerned with age-trajectories of fecundity (**2**), but Wangermann and Lacey (1955) provide data possibly indicating that fecundity declines with increasing ramet age (specifically, that the interval between successive offspring increases with offspring number) (Fig. 1-3). These data are difficult to interpret because: (a) out of seven treatment groups, only four exhibited noticeable increases in birth interval with increasing offspring number, (b) the *variance* in birth interval increased strongly with offspring number, and (c) the data reported are based on means, and only the initial sample size within each group is given. A retrospective statistical test on these data would be difficult to interpret.

The primary focus of Ashby and colleagues was on declines in offspring size with increasing parental age (**3**), and this they demonstrated clearly. In all of their studies, offspring surface area declined strongly and consistently with increasing parental age. First-offspring typically had surface areas of about 7-9 mm^2, whereas final-offspring had areas of just 1-2 mm^2 (Wangermann 1952, Ashby and Wangermann 1954). Interestingly, this parental age effect was reversible in that, starting from a small, late-produced offspring, successive generations of first-offspring would consistently increase in size until the maximum size was re-attained (Wangermann and Ashby 1951). The parental age effect also extended to offspring lifespan and reproductive output, both of which declined with increasing parental age (whether such declines were reversible, as with offspring size, is unclear; Wangerman and Ashby 1950). Claus (1972) followed up on the work of Ashby and colleagues by comparing lifespan, total reproductive output, and frond size, among

various n_i^{th}-offspring lineages, where *n* represents birth order and *i* represents the number of generations removed from an initial progenitor. For example, a 2_i^{th}-offspring lineage consists of the second offspring (2_1) of an initial progenitor, that second offspring's second offspring (2_2), etc. In general, Claus found that offspring size declined with increasing birth order, but birth order had little effect on lifespan or reproductive output. His results are difficult to interpret because birth order (*n*) and generations removed from an initial progenitor (*i*) were confounded. Specifically, the range in *i* declined strongly with increasing *n*, because taking successive generations of late-offspring is time-consuming and more prone to early termination than taking successive generations of early-offspring.

Ashby and colleagues provided strong evidence for phenotypic plasticity in lifespan in *L. minor* (**4**). Under the range of conditions examined, lifespan was generally inversely related to temperature (Wangermann and Ashby 1951) and nitrogen availability (Wangermann and Lacey 1955), positively related to day length (Wangermann 1952), and not affected by light intensity (Wangermann and Ashby 1951).

Finally, Ashby et al. (1949) compared lifespan among three different strains of *L. minor* (**5**). They list mean (±SE) lifespans for the three strains of 42 (SE not given), 41 (±0.93), and 36 (±1.30) days, respectively. Sample sizes were not given, but the observed difference in lifespan between the latter two strains is, in retrospect, very improbable under the null hypothesis of no difference, even assuming very low sample sizes (e.g. N = 10 fronds per strain).

SENESCENCE IN RAMETS

It is important to note that the above-described work on senescence in *L. minor* – as well as my own research described in subsequent chapters – concerns ramet senescence and not genet (i.e. 'clonal') senescence. Specifically, I am interested in how vital rates change with increasing ramet age, and the evolutionary pressures that shape ramet age-trajectories of survival, fecundity, and offspring quality. Therefore, throughout the remainder of this thesis, when discussing senescence in *L. minor*, I use terms like 'individual', 'plant', 'parent', 'offspring', and 'age' in reference to asexually-produced ramets.

Although the mixture of clonal and sexual reproduction adds a layer of complexity to classic evolutionary theory on senescence (e.g. Orive 1995, Pedersen 1995, Gardner and Mangel 1997), and research on senescence in clonal plants has primarily focused on genet senescence (e.g. Orive 1995, Pedersen 1995, Gardner and Mangel 1997, Ally et al. 2010), the fundamental paradox of senescence and basic tenets of evolutionary theory on senescence do still apply to ramets (e.g. Pedersen 1995). Specifically, the ramet is the basic unit of both sexual and clonal reproduction, both of which may produce additional copies of any given allele (Tuomi and Vuorisalo 1989). A decline in ramet vital rates with increasing ramet age (i.e. ramet senescence) is paradoxical in the sense that, all else being equal, a hypothetical allele that prevents ramet senescence should have greater future representation than an alternative allele that allows it. Of course, the same paradox applies to genet senescence. The resolution to the paradox of senescence – a decline in the force of natural selection with age (Hamilton 1966) – also applies both to

ramets and genets (Pedersen 1995). I do not mean to suggest that there may be no interesting differences in the evolutionary pressures or dynamics shaping senescence in ramets versus genets, or clonal versus non-clonal plants – but simply that ramet senescence falls well within the scope of classic evolutionary theory.

Figure 1-1. *L. minor* covering part of a pond in Alberta.

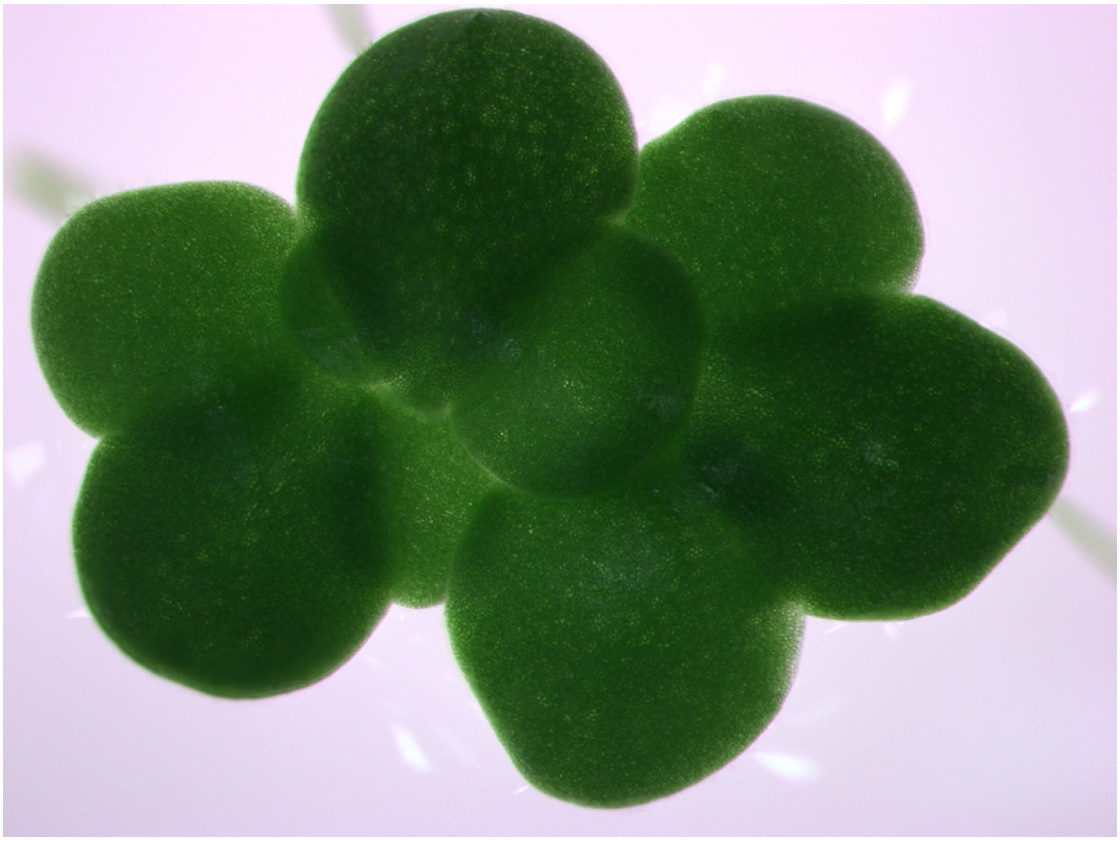

Figure 1-2. A frond of *L. minor* with daughters budding from both the right and left meristematic pockets. Each of the focal plant's daughters can be seen budding their own daughters, and the focal plant's *next* right and left daughters are already budding as well.

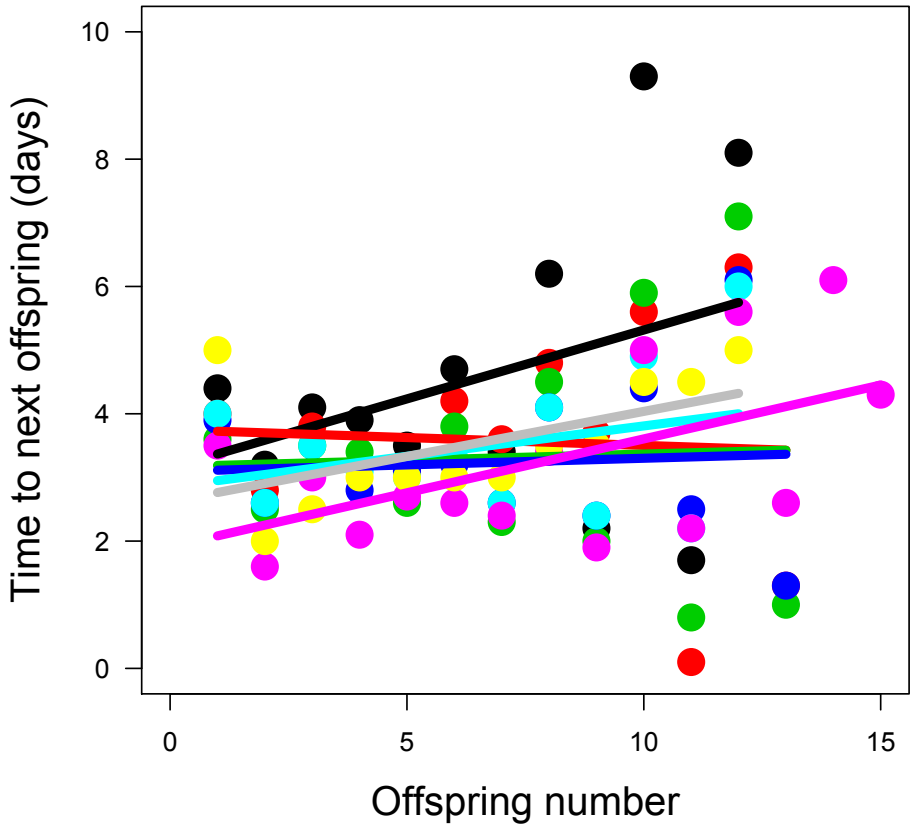

Figure 1-3. Time interval between successive offspring as a function of offspring number (i.e. inverse rate of fecundity versus age). Data are from Wangermann and Lacey (1955). Different colours represent different treatment groups relating to nitrogen concentration within the growth media.

CHAPTER 2: CHARACTERIZING SENESCENCE IN *LEMNA MINOR*

Abstract

As they grow old, most organisms experience progressive physiological deterioration resulting in declining rates of survival and reproduction – a seemingly maladaptive phenomenon known as senescence. Although senescence is usually defined with respect only to survival and reproduction, a third component of fitness, offspring quality, may also decline with age. Few studies, however, have assessed age-related changes in offspring quality using measures that truly reflect fitness. In a controlled environment, I tested for age-related declines in three demographic components of fitness (survival, reproduction, and offspring quality) in *Lemna minor*, a small aquatic plant with a short lifespan and rapid rate of asexual reproduction. My primary measure of offspring quality, the intrinsic rate of increase, more closely approximates fitness than measures used in previous studies such as size, lifespan, and total reproductive output. I observed strong age-related declines in all three components of fitness: old plants had lower rates of survival and reproduction, and produced lower-quality offspring than younger plants. Theoretical and empirical research on the evolutionary biology of senescence should devote more attention to offspring quality. This often unrecognized component of fitness may change with age – as I have shown in *L. minor* – and may be shaped by, and feed back into, the same evolutionary forces that give rise to senescence.

Introduction

Senescence is characterized by progressive physiological deterioration and age-related declines in survival and reproduction (reviewed in Kirkwood and Austad 2000, Hughes and Reynolds 2005, Williams et al. 2006, Sherratt and Wilkinson 2009). Such declines are seemingly deleterious from the perspective of an ageing individual, and yet senescence occurs in many taxa (Jones et al. 2014). Explaining the evolution and maintenance of senescence has therefore been an important challenge in evolutionary biology.

In the most general sense, the evolutionary paradox of senescence concerns age-related declines in the expectation of future genetic representation (i.e. fitness). All else being equal, a lineage that is not subject to age-related declines in fitness should have greater future representation than one that is. Although many authors define senescence with respect only to survival and reproduction, there is increasing evidence that another component of fitness, offspring quality, may also decline with age (Kern et al. 2001). For example, a decline in offspring lifespan with increasing parental age (known as the Lansing effect) has been observed in a variety of taxa including rotifers (Lansing 1947, 1948), ladybird beetles (Singh and Omkar 2009), duckweeds (Ashby and Wangermann 1949), and humans (Bell 1918, Gavrilov and Gavrilova 1997) (additional examples are cited in Priest et al. 2002). Similarly, advanced parental age has been shown to negatively affect offspring fecundity schedules in great tits (Bouwhuis et al. 2010) and pre-industrial humans (Gillespie et al. 2013a).

Age-related declines in offspring quality are paradoxical in much the same way as age-related declines in survival and reproduction. All else being equal, lineages not subject to age-related declines in offspring quality should have greater future representation than those that are. Of course, this argument is only valid insofar as offspring 'quality' reflects biological fitness. Lifespan is generally a poor measure of fitness (e.g. Jenkins et al. 2004), so despite the apparent prevalence of age-related declines in offspring lifespan, the extent to which offspring fitness declines with parental age remains unclear. Resolving this gap in our understanding is important because established theories of life history evolution and senescence implicitly assume that offspring fitness is constant with parental age (e.g. Williams 1957, Hamilton 1966, Kirkwood and Rose 1991). If this is not the case, then the force of selection cannot be understood simply in terms of age-specific survival and fecundity, but may also depend on age-specific patterns of change in offspring fitness (e.g. Pavard et al. 2007a,b, Pavard and Branger 2012, Gillespie et al. 2013a). As Caswell (2001, p. 280) points out: "The paradoxes of life history theory mean that selection must be studied in terms of the entire life cycle. The alternative – analysis in terms of a subset of vital rates, or what are called *components of fitness* – risks getting answers that are qualitatively wrong." Thus, if offspring fitness does indeed change with parental age, evolutionary analyses that ignore such changes may lead us astray.

Here I test for age-related declines in three major demographic components of fitness (survival, reproduction, and offspring fitness) in *Lemna minor*. My primary interest is to understand whether offspring fitness declines with increasing parental

age. *Lemna minor* is an excellent species in which to address this question for two reasons. First, reproduction in *L. minor* is almost exclusively asexual, which simplifies the analysis of parental age effects (there is only one parent to account for and it is easy to identify). Second, previous research suggests *L. minor* may be subject to parental-age-related declines in various offspring traits potentially relating to fitness, including offspring size, lifespan, and total reproductive output (Wangermann and Ashby 1950, 1951). Because there is a premium on early reproduction, lifespan and lifetime reproductive output may be poor measures of overall fitness (Stearns 1992, Partridge and Barton 1996). Thus, to understand whether *L. minor* is subject to age-related declines in offspring fitness (in addition to age-related declines in survival and reproduction), I employ a demographic measure that better approximates realized fitness – the intrinsic rate of increase (*r*) measured at the level of individual offspring.

Methods

OVERVIEW

I tested for age-related declines in components of fitness in two phases.

Phase one: Survival and reproduction

First, to measure the influence of age on rates of survival and reproduction, I isolated 216 fronds individually in Petri dishes containing a liquid growth medium, and observed the fronds daily for the duration of their lives. The first day of life was defined as the day that a frond detached from its parent, and death was defined as

the day that a frond's final daughter detached (there are no obvious physiological definitions of death in *L. minor*, as the progression of cell death during frond senescence generally spans 10 or more days). Every day during a frond's lifetime, I observed whether or not the frond reproduced – i.e. whether any of its daughters detached since the previous day's observation. Detached daughters were aseptically removed from the Petri dish and discarded.

Phase two: Offspring quality

The second phase of my study examined changes in offspring quality (measures included the intrinsic rate of increase, total reproductive output, latency to reproduce, lifespan, and frond size) as a function of parental age. I isolated 41 'parental' fronds individually in Petri dishes, and observed them daily for the duration of their lives, as described above. This time, however, instead of being discarded, the daughters (the 'focal' generation, N = 542) of the 41 parental fronds were transferred to their own Petri dish upon detaching from the parent, randomly assigned to one of three growth chambers, and observed for reproduction daily for the duration of their lives. Four of the 542 focal fronds (all of which were the final daughters produced by their respective parents) remained attached to their parent for a prolonged period of time – well into their reproductive lifespan. I defined the first day of life for these four individuals as the day that their first daughter detached.

PLANTS AND GROWTH CONDITIONS

The plants used in this study were derived from a clonal lineage obtained from the Canadian Phycological Culture Centre (CPCC 492 *Lemna minor*; originally collected from Elk Lake, British Columbia, Canada; 48° 31' 30" N, 123° 23' 18" W). I studied a genetically homogeneous sample because heterogeneity (both genetic and environmental) can sometimes mask true patterns of senescence (Zens and Peart 2003). Due to the possibility of parental-age effects in *L. minor* (e.g. Wangermann and Ashby 1950, 1951), I also strove for 'genealogical' homogeneity among the focal plants. Specifically, the 216 focal fronds in *Phase one* and 41 parental fronds in *Phase two* were each first daughters of first daughters (etc.) going back at least five generations.

Plants were aseptically cultured in 60 × 10 mm Petri dishes containing 10 ml of Modified Hoagland's E+ growth medium (Environment Canada 2007), and kept inside growth chambers set to 25˚C with a 12:12 photoperiod and a photosynthetic photon flux density at plant height of approximately 500 μmol m^{-2} sec^{-1}. To ensure environmental constancy (e.g. to account for evaporation, nutrient depletion, etc.), I aseptically transferred each plant into a new Petri dish with 10 ml of fresh growth medium every four days. Two of the 216 fronds from *Phase one* developed bacterial contamination and so were discarded and not included in the analyses below. There was no bacterial contamination during *Phase two*. Low rates of fungal contamination occurred in both phases of the study, always taking the form of an isolated clump of stringy white fungus within the growth medium. When such contamination was detected, the corresponding frond was aseptically transferred to a new Petri dish

with fresh growth medium. This intervention was successful given that no plant was ever subject to more than a single instance of fungal contamination.

FITNESS MEASURES

Phase one: Survival and reproduction

Measures of fitness in *Phase one* included daily rate of survival and daily rate of reproduction conditional on survival. Although fronds occasionally released two daughters on the same day (this occurred in 8.6% of the reproductive events that I observed), I chose to analyze reproduction as a binary event (0 = did not reproduce, 1 = released one or two daughters). Treating reproduction as binary instead of ordinal made it easier (statistically) to account for non-independence due to repeated observations on the same individuals.

Phase two: Offspring quality

My primary measure of offspring fitness was the intrinsic rate of increase (r) measured at the level of individual fronds, as described in McGraw and Caswell (1996). Intrinsic rate of increase is an appropriate measure of fitness for stable populations under constant environmental conditions (Metcalf and Pavard 2007), and can be calculated as the natural logarithm of the dominant eigenvalue of a Leslie matrix. To construct a Leslie matrix for single individuals, the age-specific survival rate was set to 1 for each age at which an individual survived, and 0 for every other age (McGraw and Caswell 1996). Measuring fitness in this way – at the level of the individual – is sometimes problematic due to a lack of replication (Link et al. 2002).

However, my use of a single clone negates this problem. The realized fitness of replicate fronds of a given parental age should reflect the same underlying fitness propensity (or 'latent fitness'), and thus, my approach entails appropriate replication.

In addition to the primary measure of offspring fitness (the intrinsic rate of increase), I examined four secondary measures of offspring quality (not necessarily directly related to fitness): total number of offspring produced, latency to first reproduction (days between detachment from parent and first daughter detaching; inversely related to fitness), lifespan (days between detachment from parent and last daughter detaching), and frond surface area. Frond surface area was measured in ImageJ v. 1.43u (Rasband 2012) using images captured with a microscope-mounted digital camera. Images used for surface-area measurement were captured late in a frond's life when it had no attached daughters. Occasionally, fronds produced late in their parent's life were 'curled' (see Fig 2-1), which complicated the measurement of surface area. For the 42 focal fronds in *Phase two* that were curled, I estimated surface area based on the length of each frond's longitudinal axis. These 'corrected' estimates were interpolated from a linear regression of surface area on length for the 500 non-curled fronds (Fig. 2-2).

DATA ANALYSIS

All analyses were conducted in R v. 3.1.2 (R Core Team 2015).

Phase one: Survival and reproduction

To understand how daily rates of survival varied with age, I fit and compared four candidate survival models (described in Pletcher et al. 2000, Sherratt et al. 2010): exponential, Weibull, Gompertz, and logistic. The exponential model serves as a null hypothesis of no senescence because it assumes a constant rate of survival with age, whereas survival may decline with age in the other models. All survival models were fit by maximizing log-likelihood functions using the *optim* function in R, and strength of evidence was assessed using the Akaike information criterion corrected for small sample sizes, AIC_C (Burnham and Anderson 2002).

To test for age-related declines in the daily rate of reproduction, I used generalized estimating equations (GEE) with a binomial error structure and logit link, fit with the *geeglm* function in the R package *geepack* (Halekoh et al. 2006). The GEE approach was ideal for this analysis given the possibility of within-individual negative temporal autocorrelation in reproduction (i.e. an individual that reproduces on a given day is somewhat less likely to reproduce the very next day). Due to this possibility, I favoured (based on biological relevance) a first-order autoregressive (AR-1) correlation structure, which assumes that the correlation between repeated observations on the same subject is inversely related to the distance (or time) between those observations. Other common correlation structures include 'exchangeable' (constant within-subject correlation; similar to a mixed-effects model with subject-level random intercepts) and 'independence' (no within-subject correlation; equivalent to a generalized linear model) (Zuur et al. 2009). I used the Rotnitzky–Jewell (RJ) criteria (Rotnitzky and Jewell 1990) and the rule-out criterion proposed by Shults et al. (2009) to compare the three correlation

structures described above, and a Wald test to assess the effect of age on probability of reproduction. The RJ criteria include three metrics by which to compare robust (empirical) estimates of a covariance matrix to naïve (model-based) covariance estimates. The model in which the working correlation structure best approximates the 'true' correlation structure is the model for which empirical and model-based covariance estimates are most similar (Wang and Carey 2004, Shults et al. 2009). The rule-out criterion rejects correlation structures yielding estimated covariance matrices that are not positive definite – indicative of a misspecified correlation structure (Crowder 1995, Schults et al. 2009). Note that, in the analyses of reproduction described above, I excluded data for the first day of each frond's life because none of the 216 focal fronds in *Phase one* reproduced on day one.

Phase two: Offspring quality

To understand whether offspring quality declined with parental age, I modeled the primary measure of offspring fitness (intrinsic rate of increase) and secondary measures of offspring quality (total offspring, latency to first reproduction, lifespan, and surface area) as functions of the age of the parent when the focal frond (i.e. offspring) detached, while controlling for the growth chamber that the focal frond was assigned to. All of the relationships between offspring quality and parental age were nonlinear and could not be transformed to linearity, so in all cases I examined polynomials of parental age up to a degree of three.

The modeling approach described hereafter follows Zuur et al. (2009). To account for potential non-independence of offspring derived from the same parent, I

initially fit linear mixed models describing a given measure of offspring quality as a function (either linear, quadratic, or cubic) of parental age and linear function of growth chamber, with one of three random effect structures: (i) random intercept and slope terms for parent identity, (ii) random intercept term for parent identity, or (iii) no random effects. These models were fit via restricted maximum likelihood (REML) using the *lme* or *gls* functions (*gls* was used for models without random effects) in the package *nlme* (Pinheiro and Bates 2010). To identify the best random effect structure (separately for each measure of offspring quality), I compared the nine models (3 random effect structures × 3 polynomials of parental age) using AIC_C. I did not encounter any instances in which the 'best' random effect structure differed between the three polynomials of parental age for a given measure of offspring quality (i.e. selection of the best random effect structure was always unanimous).

Once the best random effect structure was established, I moved on to the fixed effects (parental age and growth chamber). In this portion of the analysis, models were fit via maximum likelihood (ML), again using either the *lme* or *gls* functions. My approach here was to construct 'full' models describing each of the five measures of quality as a cubic function of parental age and linear function of growth chamber (with the appropriate random effect structure, as described above). I then compared all fixed-effect subsets of each full model using the *dredge* function in the package *MuMIn* (Bartoń 2013) and AIC_C values. My all-subsets approach yielded eight models for each measure of offspring quality: three polynomials of parental age (either with or without a term for growth chamber), a growth chamber only model, and a null model with only an intercept.

I visually assessed model assumptions (independent, normally-distributed error with homogeneous variance) for each measure of offspring quality using standard diagnostic plots including quantile-quantile plots, histograms of model residuals, scatterplots of residuals versus fitted values, and scatterplots or histograms of residuals versus independent variables (including the random effect term for parent identity). Diagnostic plots suggested that parametric assumptions were violated for the best model of intrinsic rate of increase (residuals were positively skewed). I therefore repeated the above-described protocol on log-transformed intrinsic rates of increase, which resulted in a best model that was more closely in line with parametric assumptions.

Results

PHASE ONE: SURVIVAL AND REPRODUCTION

I observed a significant decline in daily rates of survival with increasing frond age (Fig. 2-3a). In particular, of the four candidate survival models that I examined, the three models in which survival rates declined with age received greater statistical support (i.e. had much lower AIC_C values) than the exponential model, which assumes a constant survival rate (Table 2-1). I also observed significant age-related declines in the daily probability of reproduction (Wald test, $\chi^2 = 652.3$, df = 1, $P < 0.001$; Fig. 2-3b). Predicted daily probability of reproduction from the fitted GEE declined from 0.65 at day one to 0.28 at day thirty. The Wald test and predicted probabilities of reproduction described above were based on a GEE with autoregressive (AR-1) correlation, which was selected as a more appropriate

working correlation structure than 'independence' based on the RJ criteria (Table 2-2). The 'exchangeable' correlation structure was ruled out because it yielded an estimated covariance matrix that was not positive definite, potentially indicating a misspecified correlation structure (Crowder 1995, Schults et al. 2009). The estimate for the correlation parameter of the AR-1 model was -0.28 (± 0.02, SE), indicating moderate within-subject negative temporal autocorrelation in reproduction.

PHASE TWO: OFFSPRING QUALITY

There was a strong decline in my primary measure of offspring fitness, the intrinsic rate of increase, with increasing parental age (Fig. 2-3c). I also observed parental-age-related declines in three of the four secondary measures of offspring quality: total offspring produced, latency to first reproduction (this inverse measure of quality technically increased with parental age), and frond surface area (Fig. 2-4a,b,d). Lifespan, conversely, did not decline with increasing parental age (Fig. 2-4c).

The models of offspring quality selected as best (lowest AIC_C) were in all cases non-linear with respect to parental age. Specifically, best models always described offspring quality as either a quadratic or cubic function of parental age (Table 2-3). Except for frond surface area, best models (or a close second-best model in the case of latency to reproduction, $\Delta AIC_C = 0.1$) always included a term for growth chamber, suggesting that measures of offspring quality consistently differed among the three growth chambers that I used (Table 2-3). Excepting latency to reproduction and lifespan, best models also always included random intercept and

slope terms for parent identity, suggesting non-independence of offspring derived from the same parent (Table 2-3).

Discussion

I observed strong age-related declines in three demographic components of fitness in *L. minor*. Old plants had lower rates of survival and reproduction, and produced offspring of lower fitness than younger plants. While many species are known to experience age-related declines in at least one component of fitness, the current study is to my knowledge the first to demonstrate simultaneous age-related declines in these three major demographic components of fitness, and also one of few studies to demonstrate age-related declines in a measure of offspring quality that closely approximates fitness (see also Gillespie et al. 2013a).

OFFSPRING QUALITY AND THE EVOLUTION OF SENESCENCE

Classic theories for the evolution of senescence implicitly assume that all offspring are of equal quality, so that the action of natural selection depends only on age-specific rates of survival and reproduction (e.g. Williams 1957, Hamilton 1966, Kirkwood and Rose 1991). My results suggest that this assumption does not always hold, in which case selection may depend additionally on age-specific trajectories of offspring fitness. Why would this matter? There are few relevant theoretical results, but a recent analysis by Gillespie et al. (2013b) suggests that birth-order-related declines in offspring fitness (similar in principle to parental-age-related declines) lead to steeper declines in the force of selection compared to what would be

expected under classical models of senescence. In other words, not accounting for declining offspring fitness, where it occurs, may lead us to underestimate age-related declines in the force of selection. As many authors have argued, senescence, or more generally the action of selection, cannot be understood in terms of a single 'vital rate' or component of fitness (Partridge and Barton 1996, Caswell 2001, Nussey et al. 2008). I suggest, following Kern et al. (2001), that research on the evolutionary biology of senescence should devote attention to one extra vital rate – offspring quality. This often unrecognized component of fitness can clearly change with age, as I have shown in *L. minor*, and may be just as important in shaping overall fitness as survival and fecundity.

SENSCENCE IN PLANTS

Evolutionary theories of senescence suggest that age-related declines in fitness evolve because, for populations subject to nonzero mortality, the force of natural selection declines with age (Medawar 1952, Williams 1957, Hamilton 1966). Simply put, natural selection discounts old age-classes because relatively few individuals survive into old age, even in the absence of senescence. However, a number of authors have suggested that senescence should be relatively rare among vascular plants (Vaupel et al. 2004, Peñueles and Munné-Bosch 2010) or even that plants are predisposed to immortality (Silvertown et al. 2001). Such views are based on unique aspects of the plant form and life history. For example, unlike other organisms that exhibit determinate growth, many vascular plants exhibit continual growth and regeneration via totipotent apical meristems (Roach 2003). This

indeterminate growth pattern potentially allows for a continual increase in reproductive potential with age, which may translate into an increase in the force of natural selection with age (Vaupel et al. 2004).

Although some iteroparous plants (e.g. Herrera and Jovani 2010, Shefferson and Roach 2013) and all semelparous plant species exhibit senescence, comparative studies to date have largely confirmed the predicted rarity of senescence among iteroparous vascular plants (Silvertown et al. 2001, Baudisch et al. 2013). Furthermore, a recent analysis by Caswell and Salguero-Gómez (2013) found that the force of selection does in fact increase with age for many iteroparous plants, especially within later stages of the plant life cycle. Why then is the iteroparous *L. minor* subject to senescent decline when its relatives within Plantae seem mostly immune? Unlike most vascular plants, *L. minor* has a unitary growth form and exhibits determinate growth at the level of individual fronds, which usually reach their full growth potential prior to detaching from their parent (Hillman 1961). Without indeterminate growth and continually increasing reproductive potential, the force of natural selection is generally expected to decline with age (Hamilton 1966), making *L. minor*'s age-related declines in fitness components consistent with evolutionary theory.

SENESCENCE IN *LEMNA*

Wangermann and Ashby (1950, 1951) documented parental-age-related declines in offspring size, lifespan, and lifetime reproductive output in *L. minor*, whereas Claus (1972) observed a slight increase in offspring lifespan and no change

in lifetime reproductive output with increasing birth order (similar in principle to parental age). In Claus's study, birth order was confounded with other aspects of genealogy and there were very few plants representing the highest birth orders (i.e. greatest parental ages), so his results are difficult to interpret and I do not consider them further. Similar to Wangermann and Ashby, my results demonstrate age-related declines in offspring size and lifetime reproductive output, and I extend the results of Wangermann and Ashby in a manner relevant to evolutionary theories of senescence by specifically demonstrating age-related declines in offspring fitness (i.e. intrinsic rate of increase). I did not, however, observe declines in offspring lifespan with increasing parental age. One possible explanation for the conflicting results relates to how I defined death (i.e. the day that a frond's final daughter detached). It is not clear to me exactly how Wangermann and Ashby defined death, but they seem to have assessed death visually based on a loss of pigment. The difference between these two definitions of death might be considered the post-reproductive lifespan (i.e. the time between the final reproductive event and the complete loss of pigment). If post-reproductive lifespans (but not reproductive lifespans) tend to decline with increasing parental age in *L. minor*, I would expect to see age-related declines in offspring lifespan under Wangermann and Ashby's (presumed) definition of death, but not under my own.

PROXIMATE EXPLANATIONS FOR DECLINING OFFSPRING QUALITY

Age-related declines in offspring quality (i.e. parental age effects) have been observed in a wide range of taxa, and a number of hypotheses have been put forth

concerning the proximate cause of such declines. My thesis is not specifically concerned with proximate causes of parental age effects, but some results from this and subsequent chapters relate to proximate causation nonetheless. I discuss this topic further in Chapter 6.

CONCLUSIONS

I found that, in a controlled laboratory environment, *L. minor* fronds exhibited age-related declines in three major demographic components of fitness – survival, reproduction, and offspring quality. Following Kern et al. (2001), I suggest that both theoretical and empirical research on the evolutionary biology of senescence should devote more attention to age-related changes in offspring quality. This often unrecognized component of fitness can clearly change with age, as I have shown in *L. minor*, and may be just as important in shaping overall fitness as survival and fecundity. Incorporating offspring quality into demographic and evolutionary analyses will no doubt be challenging. Indeed, determining the appropriate measure of fitness is difficult even when only the traditional fitness components – survival and fecundity – are considered (Link et al. 2002, Metcalf and Pavard 2007). Nonetheless, treating offspring quality as a component of fitness that may covary or trade-off with other fitness components, and be shaped by age-specific changes in the force of natural selection alongside other fitness components, may provide important insight into the evolutionary biology of senescence.

Table 2-1. Comparison of models describing age-specific rates of frond survival. The best model (lowest AIC_C) is in bold.

Model	Parameters	Deviance	AIC_C	ΔAIC_C	AIC_C weight
Logistic	**3**	**1195.9**	**1202.0**	**0.0**	**0.99**
Weibull	2	1222.1	1226.2	24.2	<0.001
Gompertz	2	1258.5	1262.5	60.5	<0.001
Exponential (no senescence)	1	1808.1	1810.1	608.1	<0.001

Table 2-2. Comparison of working correlation structures for GEE models describing age-specific rates of reproduction. The 'best' working correlation structure (in bold) is the one that yields values of RJ1 and RJ2 closest to 1, and a value of RJ3 closest to 0. Working correlation structures that fail to yield a positive definite covariation matrix are ruled out.

Working correlation structure	Positive definite covariation matrix?	RJ1 ($\bar{c}_1$)	RJ2 ($\bar{c}_2$)	RJ3 ($\bar{d}$)
Independence	yes	0.25	0.07	0.58
Autoregressive (AR-1)	**yes**	**0.42**	**0.19**	**0.36**
Exchangeable	no	–	–	–

Table 2-3. Comparison of models describing measures of offspring quality as functions of parental age (par) and growth chamber (chamb). For each measure of quality, the best model (lowest AIC_C) is in bold. Only the five best models are displayed for each measure of offspring quality.

Measure of offspring quality	Model[a]	df	Deviance	AIC_C	ΔAIC_C	AIC_C weight
ln (Intrinsic rate of increase)[c]	**par^2 + chamb**	**9**	**-192.8**	**-174.4**	**0**	**0.44**
	p^2	7	-187.8	-173.6	0.9	0.29
	par^3 + chamb	10	-192.9	-172.5	2.0	0.17
	par^3	8	-187.9	-171.7	2.8	0.11
	par^1	6	-127.4	-115.2	59.2	<0.001
Total offspring[c]	**par^3 + chamb**	**10**	**1854.1**	**1874.5**	**0**	**0.83**
	p^3	8	1861.4	1877.6	3.1	0.17
	par^2 + chamb	9	1871.0	1889.3	14.9	<0.001
	par^2	7	1877.0	1891.2	16.7	<0.001
	par^1 + chamb	8	1963.0	1979.3	104.8	<0.001
Latency to reproduce[b]	**par^2**	**4**	**1502.4**	**1510.5**	**0**	**0.30**
	par^2 + chamb	6	1498.4	1510.6	0.1	0.28
	par^3	5	1501.0	1511.1	0.6	0.22
	par^3 + chamb	7	1497.1	1511.3	0.8	0.20
	par^1	3	1571.7	1577.8	67.3	<0.001
Lifespan[b]	**par^3 + chamb**	**7**	**3005.0**	**3019.2**	**0**	**0.95**
	par^2 + chamb	6	3013.0	3025.2	6.0	0.05
	chamb	4	3025.2	3033.3	14.1	0.001
	par^1 + chamb	5	3025.2	3035.3	16.1	<0.001
	par^3	5	3033.2	3043.3	24.1	<0.001
Frond surface area[c]	**par^3**	**8**	**970.2**	**986.5**	**0**	**0.67**
	par^3 + chamb	10	967.5	987.9	1.4	0.34
	par^2	7	1002.0	1016.2	30.0	<0.001
	par^2 + chamb	9	998.4	1016.7	30.2	<0.001
	par^1	6	1236.4	1248.5	262.0	<0.001

a. Numeric superscripts beside the parental age term (par) indicate polynomial degree
b. Models do not include random effects
c. Models include random intercept and slope terms for parent identity

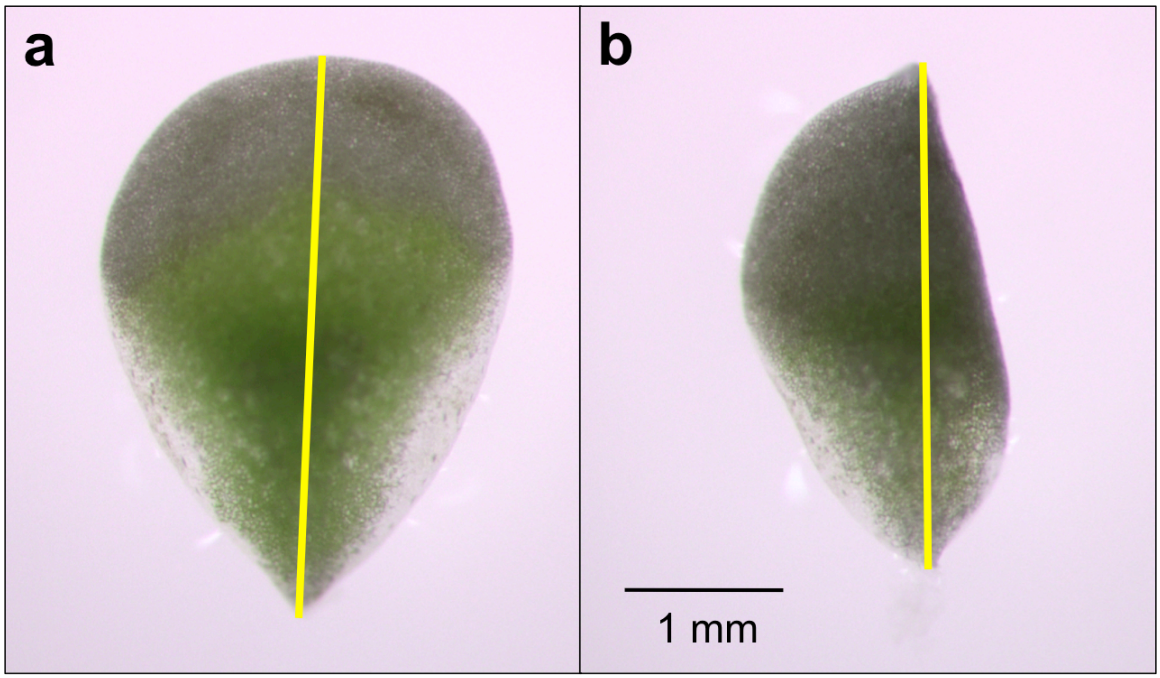

Figure 2-1. Comparison of non-curled (**a**) and curled (**b**) fronds. Yellow lines correspond to each frond's longitudinal axis. I used the strong correlation between surface area and length of the longitudinal axis to estimate the surface area of the 42 (out of 542) fronds in *Phase two* that were curled (see also Fig. 2-2). Note that the 1 mm scale bar applies to both panels.

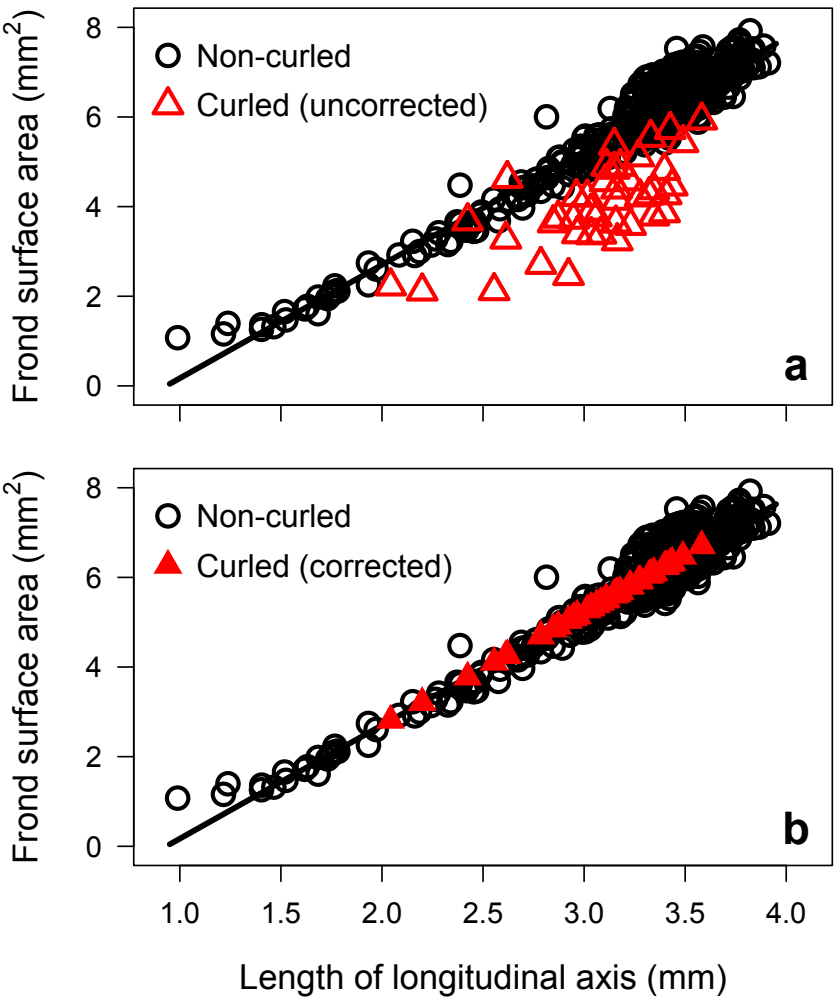

Figure 2-2. Linear regression of frond surface area (mm^2) versus length of the longitudinal axis (mm) for the 500 non-curled fronds (open black circles) in *Phase two*. Uncorrected (open red triangles, panel **a**) and corrected (filled red triangles, panel **b**) surface areas of curled fronds are depicted for comparison. I 'corrected' estimates of surface area for curled fronds by interpolating from the regression line (which was fit using data from the 500 non-curled fronds).

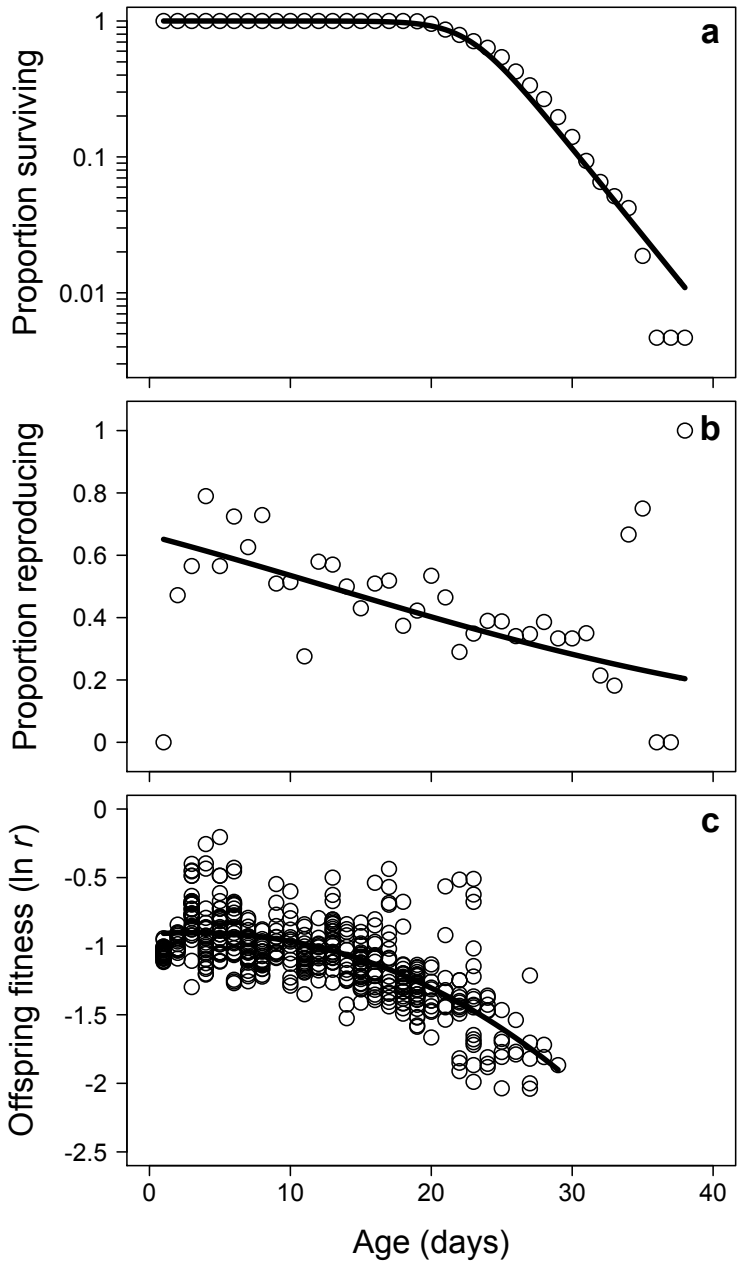

Figure 2-3. Age-related declines in rates of survival (**a**), rates of reproduction (**b**), and offspring fitness (**c**) in *L. minor*. Offspring fitness was measured as the ln-transformed intrinsic rate of increase (r), which has units of day^{-1}. Best-fit models are described in the text and Tables 2-1 to 2-3. In semi-log survival plots such as in **(a)**, a population with constant survival rates (i.e. with no senescence) would appear as a straight line.

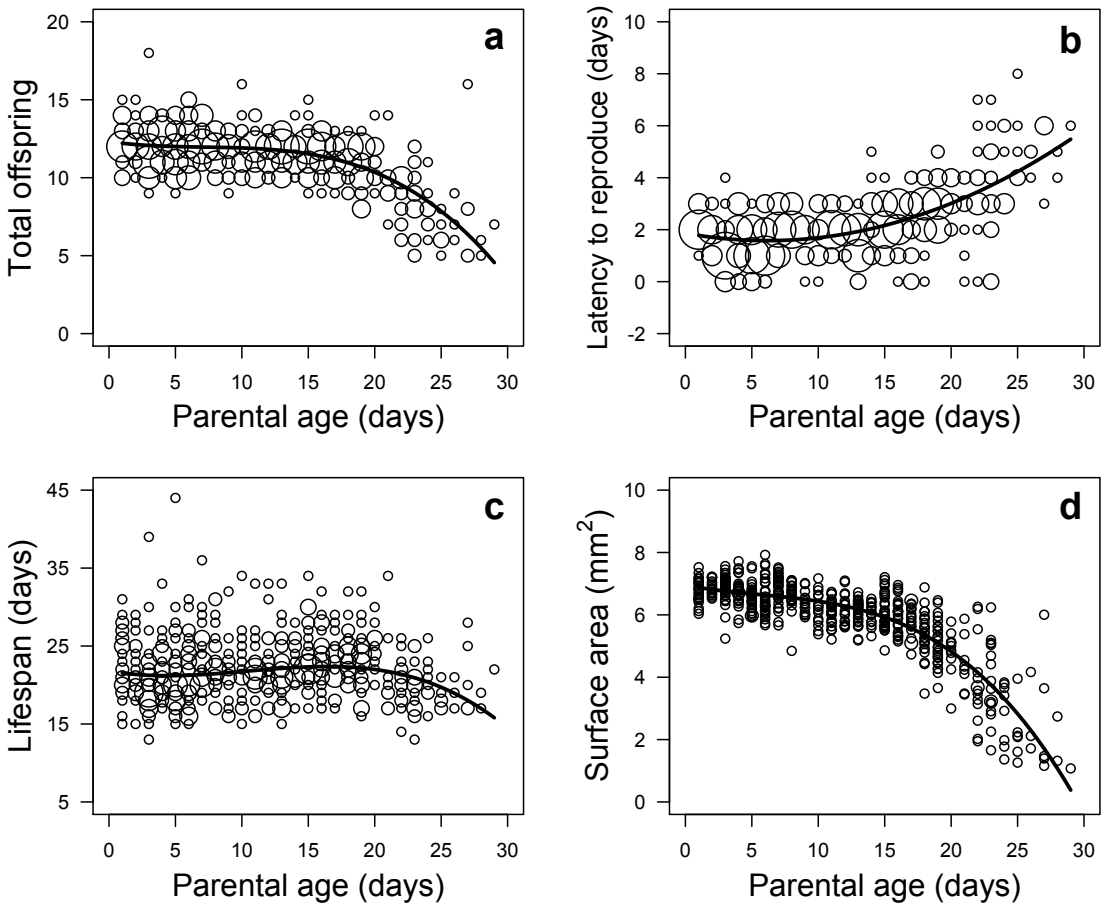

Figure 2-4. Parental-age-related changes in secondary measures of offspring quality including total offspring produced (**a**), latency to first reproduction (inversely related to fitness) (**b**), lifespan (**c**), and frond surface area (**d**). Point area is proportional to the number of observations at a given set of coordinates. Best-fit models are described in the text and Table 2-3.

CHAPTER 3: DO PARENTAL AGE EFFECTS IN *LEMNA MINOR* EXTEND OVER MULTIPLE GENERATIONS?

Abstract

Classic theories on the evolution of senescence make the simplifying assumption that all offspring are of equal quality, so that demographic senescence only manifests through declining rates of survival or fecundity. However, there is a great deal of evidence for age-related declines in offspring quality (i.e. parental age effects) in a wide range of taxa. Recent modeling approaches allow for the incorporation of parental age effects into classic demographic analyses, assuming that such effects are limited to a single generation. Does this 'single-generation' assumption hold? To find out, I conducted a laboratory study with the aquatic plant *Lemna minor*, a species for which parental age effects have been demonstrated previously. I compared the size and fitness of 423 lab-cultured plants representing various birth orders (a proxy for parental age), and ancestral 'birth-order genealogies'. I found that offspring size and fitness both declined with increasing 'immediate' birth order (i.e. birth order with respect to the immediate parent), but only offspring size was affected by ancestral birth order. Thus, the assumption that parental age effects on offspring fitness are limited to a single generation does in fact hold for *L. minor*.

Introduction

Age-related declines in physiological and demographic performance (known as ageing or senescence) seem inherently maladaptive, but occur nonetheless in many taxa (Jones et al. 2014). Evolutionary theorists have proposed a variety of mechanisms to explain this apparent paradox (e.g. mutation accumulation, Medawar 1946, 1952; antagonistic pleiotropy, Williams 1957; disposable soma, Kirkwood 1977, Kirkwood and Holliday 1979; reliability theory, Gavrilov and Gavrilova 2001, Laird and Sherratt 2009, 2010), all centered around the realization that the force of natural selection tends to decline with increasing age (Hamilton 1966). Simply put, natural selection discounts relatively old age-classes because, assuming any nonzero level of mortality, fewer and fewer individuals survive to increasingly advanced ages.

One simplifying assumption implicit in the majority of theoretical work on senescence is that all offspring are of equal quality (e.g. Hamilton 1966, Kirkwood and Rose 1991, Vaupel et al. 2004). Under this assumption, fitness and the force of natural selection depend on age trajectories of only two fitness components – survival and fecundity. Thus, senescence, from an evolutionary perspective, is generally defined as a decline in the rate of survival or fecundity (or both) with increasing age. As others have pointed out (Kern et al. 2001), this view of senescence omits age-related declines in offspring quality (i.e. parental age effects), which are known to occur in a wide range of taxa (Priest et al. 2002, Descamps et al. 2008, Bouwhouis et al. 2010, Gillespie et al. 2013b, Barks and Laird 2015). Recent analyses suggest that, if offspring quality does in fact decline with increasing parental age,

classic demographic methods that consider only survival and fecundity may incorrectly estimate age-related declines in the force of selection (Pavard et al. 2007a,b, Pavard and Branger 2012, Gillespie et al. 2013a).

In Chapter #2, I demonstrated parental-age-related declines in offspring quality in *L. minor*. Specifically, I found that offspring produced late in their parent's life were smaller and had lower fitness than their earlier-produced siblings. To fully understand how variation in offspring quality influences the force of natural selection, we need to understand not only how offspring quality changes with parental age, but also whether effects of parental age carry over across multiple generations. For example, Hercus and Hoffman (2000) found that, in *Drosophila serrata*, offspring fitness declined both with increasing maternal age and increasing grandmaternal age (the age of the grandmother at the mother's birth). Intuitively, natural selection should discount old age-classes if individuals within those classes tend to produce offspring of relatively low quality. This discount should be especially large if the negative effects of old age carry over across multiple generations.

Here I ask: do parental-age-related declines in offspring quality in *L. minor* carry over across multiple generations? There is some evidence to suggest that they do, at least in terms of offspring size (one aspect of quality). Specifically, Wangermann and Ashby (1951) found that late-produced offspring in *L. minor* were much smaller than their earlier-produced siblings. Moreover, these small, late-produced plants themselves produced relatively small first-offspring compared to earlier-produced plants, suggesting a grandparental age effect on offspring size. In

the current study, I extend the work of Wangermann and Ashby (1951) by examining variation in a measure of offspring quality more closely related to fitness (the intrinsic rate of increase), over a wider range of parental and ancestral ages.

Methods

OVERVIEW

To test whether parental age effects carry over across multiple generations, I sought to compare the fitness (measured as the intrinsic rate of increase) of 512 focal plants comprising 16 'birth-order genealogies' (Fig. 3-1). Birth order is a proxy for parental age reflecting the temporal order in which the offspring of a given parent are born. Specifically, an individual with birth order N is the Nth offspring born to its parent. In *L. minor*, parents have two meristematic pockets (right and left) from which offspring may detach, so I define N_P as the pocket-specific birth order where P can either be right ('R') or left ('L'). For example, a plant with birth order N_R is the N_Rth offspring to detach from the right meristematic pocket of its parent. Because, in *L. minor*, offspring develop alternately between the two meristematic pockets, a plant with birth order N_P will generally have an overall (pocket-independent) birth order of $N = 2 \times N_P$ or $N = 2 \times N_P - 1$, depending on which pocket produced the first offspring. To limit potential heterogeneity in my sample, I studied right-produced offspring only, with exceptions noted below.

In the current study, the birth-order genealogy of each focal plant was captured by two variables: immediate birth order and ancestral birth order. Immediate birth order was the birth order of a focal plant with respect to its parent

(target values in my study were N_R = 1, 3, 5, or 7), whereas ancestral birth order reflected birth order over the three preceding generations (target values were N_R-N_R-N_R = 1-1-1, 3-3-3, 5-5-5, or 7-7-7). Previous research documented declines in offspring size and fitness with increasing *immediate* birth order in *L. minor* (Wangermann and Ashby 1950, Barks and Laird 2015). If parental age effects carry over across multiple generations, then I expect frond size and fitness to decline with increasing *ancestral* birth order as well.

PLANTS AND GROWTH CONDITIONS

The single strain of *L. minor* used in this study was initially collected from a small wetland at the University of Lethbridge in Lethbridge, Alberta (49.6792°N, 112.8726°W). From these wild-collected plants I created a sterile, single-genotype stock culture following the protocol described in Hillman (1961), as further detailed in Appendix 2. The stock was maintained in half-strength Schenk and Hildebrandt (S-H) growth medium (Sigma-Aldrich S6765), which I supplemented with sucrose (6.7 g/L), yeast extract (0.067 g/L), and tryptone (0.34 g/L) to make microorganism contamination more easily detectable.

Except for the stock culture, plants used in this study were grown individually in 60 × 10 mm Petri dishes containing 10.5 mL of S-H medium (supplemented as described above). Petri dishes were arranged on cookie-cooling racks and kept inside growth chambers at 24˚C with a 15:9 photoperiod and photosynthetic photon flux density at plant height of approximately 400 μmol m^{-2} s^{-1}. To account for nutrient depletion and evaporation of the growth medium, plants

were aseptically transferred to new Petri dishes containing fresh growth medium every four days.

PLANT OBSERVATION

To create the 16 genealogical sequences (4 immediate × 4 ancestral birth orders) and measure the fitness of focal fronds, I had to keep track of reproduction by individual plants on a daily basis. This daily tracking regime began with 32 progenitor fronds initially taken from the stock culture ('P' in Fig. 3-1), and continued until all focal fronds were deceased. During each daily observation period (i.e. census), I noted how many daughters had detached from each meristematic pocket of each parent since the previous census, and updated a tally of the number of daughters detached from each meristematic pocket of each parent since birth. Detached daughters were aseptically removed from the Petri dish and discarded if they were not needed, or transferred to their own fresh Petri dish if they were of the requisite birth order to continue my planned genealogical sequence (see Fig. 3-1).

MEASURING FROND FITNESS AND SIZE

I estimated the fitness of focal fronds using the individual intrinsic rate of increase (McGraw and Caswell 1996), which gives the expected rate of population increase (fronds per frond per day) in the lineage hypothetically descending from a particular focal frond, assuming that all descendants have the same lifespan and fecundity schedule as their focal frond ancestor. This metric is well suited for combining survival and fecundity schedules into a single value that can be used to

compare relative contributions to future generations across different subsets of a population.

To calculate individual intrinsic rates of increase, I created a $\omega \times \omega$ Leslie matrix for each focal frond, where ω was the frond's reproductive lifespan in days. Each matrix was populated with age-specific fertilities (F_i) across the top row (number of daughters released while in age-class i), age-specific survival probabilities (S_i) on the subdiagonal (survival was set to 1 for each age-class through which the focal frond survived), and all other elements were set to zero. Intrinsic rates of increase were then calculated as the natural logarithm of the dominant eigenvalue of each Leslie matrix (McGraw and Caswell 1996).

One difficulty associated with the individual intrinsic rate of increase is its sensitivity to the length of time between when offspring are born and when they are counted (Brommer et al. 2002). For example, if a frond is first observed to have detached from its parent at census b (the birth census), I only know that it detached sometime between censuses $b - 1$ and b. If the frond detached immediately after census $b - 1$, then its first age-class is best defined as the period between censuses $b - 1$ and b (definition #1; post-breeding census). In contrast, if the frond detached immediately before census b, then its first age-class is best defined as the period between censuses b and $b + 1$ (definition #2; pre-breeding census). In the current study, I could never be sure which definition of the first age-class was more appropriate for any given focal frond (this uncertainty applies to all demographic studies on organisms that do not reproduce in uniformly-spaced pulses). I

incorporated this uncertainty into my analysis using multiple imputation, as described in the *Data analysis* section.

Frond surface area was measured in ImageJ v. 1.43u (Rasband 2012) based on images captured with a microscope-mounted digital camera. When a frond has daughters attached, it can be difficult to delineate that frond's perimeter. I therefore captured images for surface area measurement late in each focal frond's life when it had no attached daughters.

SAMPLE LOSS AND SKIPPED CENSUSES

In creating the 16 birth-order genealogies, offspring with birth order $N_R = 7$ were sometimes difficult to obtain because fronds of relatively high birth order occasionally develop in a 'curled', deformed manner (Lemon and Posluszny 2000, Barks and Laird 2015), which can make it difficult to track the birth order and total number of their offspring (i.e. it can be difficult to distinguish left from right daughters, or daughters from granddaughters). Additionally, parents do not always produce ≥7 offspring from each meristematic pocket (though this was relatively rare in my study compared to the 'curling' described above). If a required $N_R = 7$ was not produced or appeared too deformed to reliably track, I attempted to retain its $N_R = 6$, $N_L = 7$, or $N_L = 6$ sibling instead (with preference given in that order). In a few cases where a required $N_R = 5$ was too deformed to reliably track, I retained its $N_L = 5$ sibling instead. Such swaps were not possible when the relevant siblings had already been discarded by the time it was realized that the target frond could not be reliably tracked. Thus, if I could not track a frond's reproduction with certainty and a swap

was not possible, the lineage was discontinued resulting in sample loss. Though I aimed for 512 focal plants, I was only able to successfully track 423 focal fronds to their death. As expected, sample loss increased with both immediate and ancestral birth order (Fig. 3-2).

Over an 8-day period toward the end of the study, extraneous circumstances resulted in focal fronds being observed every second or third day instead of daily. Because my fitness metric was derived from the complete reproduction schedule of each focal frond, the skipped observation periods add a small degree of uncertainty to fitness estimates for those focal fronds that were still alive during the 8-day period in question (96 of the 423 focal fronds were affected). I deal with this uncertainty using multiple imputation, as described below.

DATA ANALYSIS

All analyses were conducted in R v. 3.1.2 (R Core Team 2015). As previously mentioned, fitness estimates for some of the focal fronds were subject to uncertainty due to skipped censuses, and fitness estimates for all fronds were subject to uncertainty regarding the most appropriate definition of the first age-class. I explicitly accounted for both sources of uncertainty using multiple imputation – generating multiple simulated datasets where missing values are stochastically replaced with plausible values (outlined in Schafer 1999, Nakagawa and Freckleton 2008). Each imputed dataset is analyzed using standard methods (general linear models in my case), and parameter estimates are then 'pooled' to account for the variance both within and among datasets. Hypothesis testing on pooled parameter

estimates can be accomplished with a Wald-type test statistic D_m, as described in Meng and Rubin (1992). I generated m = 10 simulated datasets (the generally-recommended range for m is 3-10; Rubin 1987, Nakagawa and Freckleton 2008) using my own imputation algorithm (described below), and used the *pool* and *pool.compare* functions within the R package *MICE* (van Buuren and Groothuis-Oudshoorn 2011) to pool parameter estimates and obtain test statistics and p-values. I used the above-described protocol for my main hypothesis test on fitness versus immediate and ancestral birth order, and also for post-hoc contrasts following from the main test. Diagnostic plots generated for a subset of imputed datasets suggested that parametric assumptions were consistently violated (residuals were positively skewed), so I ln-transformed the intrinsic rates of increase, which consistently improved the normality of residuals. I applied the Bonferroni correction during post-hoc testing to limit Type I error rates.

The two sources of uncertainty in my analysis were constrained in that 'missing' entries logically could only take on one of two or three possible values. Specifically, I considered only two possible definitions of the first age-class (pre-breeding or post-breeding census), and I never skipped more than two sequential censuses for a given focal frond (so the range of uncertainty in an offspring's date of birth was at most three days). In each imputation, for each focal frond, I randomly and with equal probability assigned one of the two possible definitions of the first age-class. Likewise, in each imputation, for each daughter of a focal frond observed to have detached during a census immediately following one or more skipped censuses, I randomly assigned the daughter to one of the two or three possible

parental age-classes, selected with equal probability (see example in Table 3-1). Note that my imputation step did not directly generate intrinsic rates of increase *per se*, but rather stochastically generated a portion of the information used to subsequently calculate a focal frond's individual intrinsic rate of increase.

Testing the effect of birth order on frond size did not require imputation since skipped observation periods did not add any uncertainty to estimates of frond size. Thus, I assessed the effect of immediate and ancestral birth order on frond size using analysis of variance (ANOVA) and post-hoc Tukey's tests. I again used standard diagnostic plots to confirm that parametric assumptions were met.

Results

Offspring size was significantly affected by both immediate ($F_{3,413} = 99.9$, $p < 0.001$) and ancestral ($F_{3,413} = 43.5$, $p < 0.001$) birth order, whereas offspring fitness was affected by immediate birth order ($D_m = 14.3$, df = 3, 345, $p < 0.001$) but not ancestral birth order ($D_m = 0.4$, df = 3, 170, $p = 0.8$). Offspring size and fitness both peaked at an immediate birth order of $N_P = 3$, and declined with increasing immediate birth order thereafter (Figs. 3-3 and 3-4). Similarly, offspring size peaked at ancestral birth order N_P-N_P-N_P = 3-3-3 and declined thereafter (Fig. 3-4).

Uncertainty in fitness estimates due to the differing age-class definitions and skipped censuses (i.e. variation among imputations; Fig. 3-3 bottom) was small compared to variation in fitness within imputations (Fig. 3-3 top).

Discussion

My results suggest that, in *L. minor*, parental age effects on offspring size carry over across multiple generations, but parental age effects on offspring fitness (measured as the individual intrinsic rate of increase) do not. Specifically, despite offspring fitness declining with increasing immediate birth order (recall that birth order was a proxy for parental age), the fitness of focal fronds was unrelated to the birth order of their three closest ancestors. At least in *L. minor*, parental age effects on offspring fitness seem to 'reset' at each new generation.

EVOLUTIONARY CONSEQUENCES OF PARENTAL AGE EFFECTS

Intuitively, a parental age effect that is limited to a single generation should be much simpler to model than one that carries over or accumulates across generations. For instance, to incorporate single-generation parental age effects into classic population projection analyses (e.g. van Groenendael et al. 1998), we should only need to track one additional variable: parental age at birth. In other words, instead of examining population-averaged age-trajectories of survival and fecundity, we could separate age-trajectories of survival and fecundity by parental age. This was the general approach used by Pavard and colleagues (Pavard et al. 2007a, 2007b, Pavard and Branger 2012) to examine the effect of maternal care on the evolution of human life-history traits. In their models, offspring survival to maturity depended on maternal survival, the probability of which declined with increasing maternal age. In general, Pavard and colleagues found that incorporating the above-described maternal effect into population projection analyses resulted in an increased force of selection on adult (maternal) survival, and an increased rate of

decline in the force of selection on maternal fecundity, compared to what was expected if maternal effects were ignored. In principle, it should be possible to extend this approach to examine parental age effects on adult traits (both survival and fecundity), like the effects I observed in *L. minor*. Because, in *L. minor*, offspring fitness depends on parental age but not parental survival *per se* (as it does in humans and other animals with parental care), I predict that the incorporation of parental age effects into demographic models for *L. minor* should generally lead to a relatively steeper decline in the age-specific force of selection on both adult survival and fecundity. There will be little selection to survive and reproduce at advanced ages if offspring produced at those ages are inherently of low quality.

PROXIMATE CAUSES OF PARENTAL AGE EFFECTS

The current study was not specifically concerned with the proximate causes of parental age effects, but some of my results may pertain to proximate causation nonetheless. I discuss this topic further in Chapter 6.

CAVEATS

There was a relatively high rate of missing data in the current study (I aimed for 512 focal plants but only successfully tracked 423 to their death), and the rate of missingness increased with both immediate and ancestral birth order (Fig. 3-2). Could this pattern of missing data have significantly biased my results? I think it unlikely. In the current study, samples were primarily lost when fronds (generally of high birth order) developed in a 'curled' manner and could not be reliably tracked. If,

for a given birth order, fronds that are curled consistently have higher (lower) fitness than non-curled fronds, then my study may have underestimated (overestimated) the decline in offspring fitness with increasing birth order. As far as I can tell, whether or not a frond is curled has little bearing on its fitness. In Chapter 2, I was able to track the reproduction of curled fronds over the duration of their lives (in that study I used a different genetic strain, and all fronds had ancestral birth order N_P-N_P-N_P-N_P = 1-1-1-1). Data from Chapter 2 indicate that, for a given parental age, curled and non-curled fronds have similar fitness (Fig. 3-5).

CONCLUSIONS

A recently-developed modeling approach (Pavard et al. 2007a,b, Pavard and Branger 2012) allows for the incorporation of parental age effects into classic population projection analyses, assuming that the parental age effects are limited to a single generation. My results suggest that this assumption holds in *L. minor*, at least with respect to a composite measure of offspring fitness – the individual intrinsic rate of increase. Whereas Pavard and colleagues' work was based on maternal age effects on juvenile traits, the parental age effect I observed in *L. minor* affected adult traits (there is no juvenile period in *L. minor*), and thus may modify the force of selection in ways that have yet to be described. Following Kern et al. (2001), I suggest that an increased incorporation of parental age effects into evolutionary theory on senescence will further our understanding of the selective forces that have led to the remarkable diversity in patterns of senescence that exists in nature.

Table 3-1. Hypothetical example of the six possible imputations for a scenario in which, for a given focal frond, two sequential censuses are skipped, followed by a third census at which two daughters are observed to have detached. Values of age-specific fecundity (F_i) for which there is uncertainty due of the skipped censuses are shaded grey. For each of the six possible imputations, I depict the corresponding individual intrinsic rate of increase (r) in the rightmost column, assuming that the hypothetical focal frond did not reproduce beyond age class 6.

	F_1	F_2	F_3	F_4	F_5	F_6	r
Observed	1	0	skipped	skipped	2	1	
Imp. 1	1	0	2	0	0	1	1.73
Imp. 2	1	0	0	2	0	1	1.59
Imp. 3	1	0	0	0	2	1	1.51
Imp. 4	1	0	1	1	0	1	1.66
Imp. 5	1	0	1	0	1	1	1.62
Imp. 6	1	0	0	1	1	1	1.55

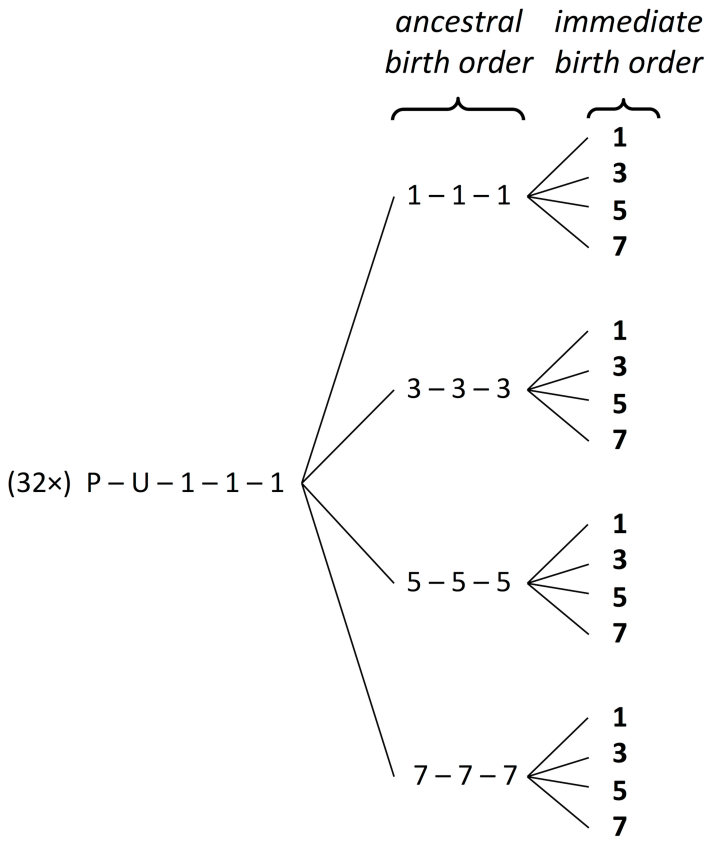

Figure 3-1. Schematic of the 16 birth-order genealogies. The leftmost element represents the earliest-tracked ancestors and the rightmost elements represent focal fronds. Numbers represent the pocket-specific birth order (N_P) of a given frond with respect to its immediate parent. The 'P' at the far left of the schematic represents one of 32 progenitor fronds initially taken from a stock culture, and the adjacent 'U' represents the progenitor's first observed offspring, which is always of unknown birth order because the progenitor may have released offspring while still in the stock culture. The birth-order genealogy of each focal frond is captured by two variables: immediate birth order (birth order with respect to the immediate parent), and ancestral birth order (birth order over the three preceding generations).

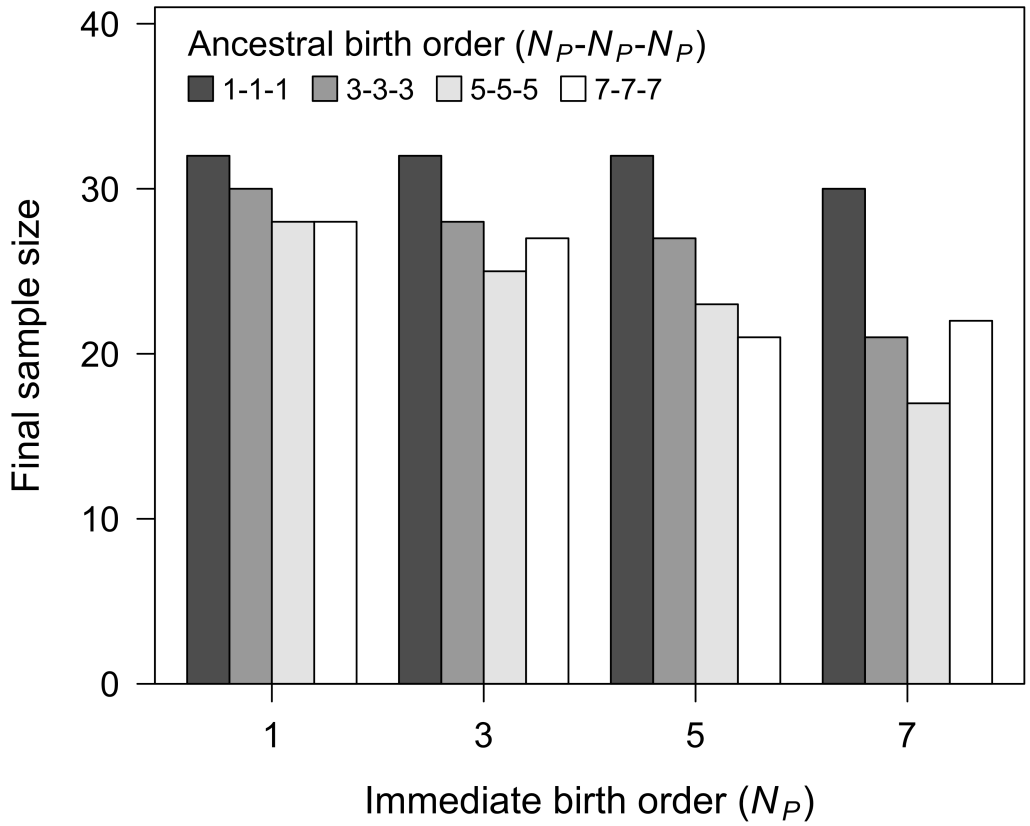

Figure 3-2. Final sample size (number of focal fronds) for each of the 16 birth-order genealogies. Samples were lost when a frond failed to produce a daughter of high enough birth order to continue the planned genealogical sequence, or when a frond's reproduction could not be tracked with certainty (due to the frond developing in a curled manner, as described in the text).

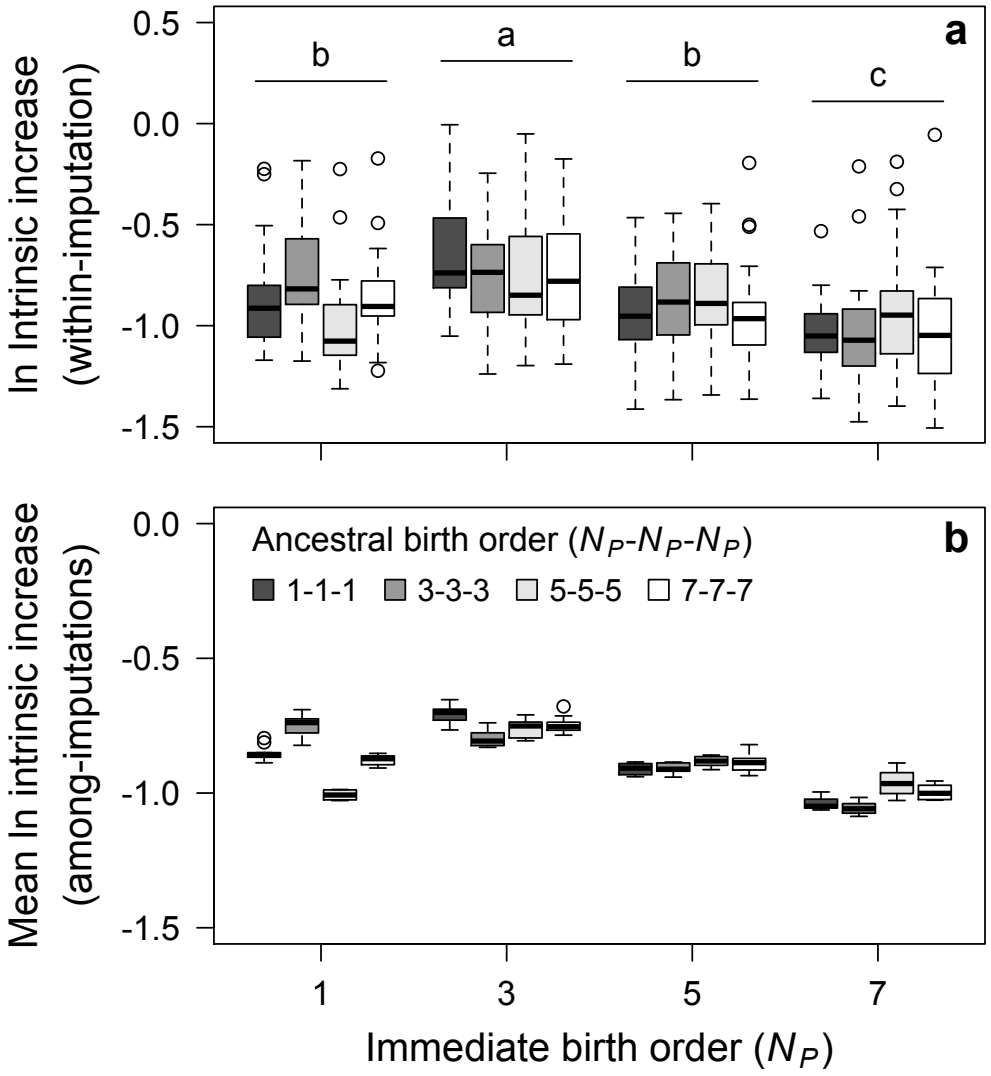

Figure 3-3. ln-Transformed individual intrinsic rates of increase by immediate and ancestral birth order. Panel **a** depicts intrinsic rates of increase for one of the 10 imputed datasets, whereas panel **b** depicts variation in mean intrinsic rates of increase *among* the 10 imputed datasets. Note that the range of the y-axis is smaller in panel **b** (for greater clarity), and even so, variation within imputations (top panel) is visibly much greater than variation among imputations (bottom panel). Letters above the boxplots indicate significant differences among immediate birth orders based on Bonferroni-corrected post-hoc contrasts. There was no significant effect of

ancestral birth order on intrinsic rates of increase. Boxes depict the median and first and third quartiles, and whiskers extend to the lowest and highest data points within 1.5 times the interquartile-range of the first and third quartile, respectively.

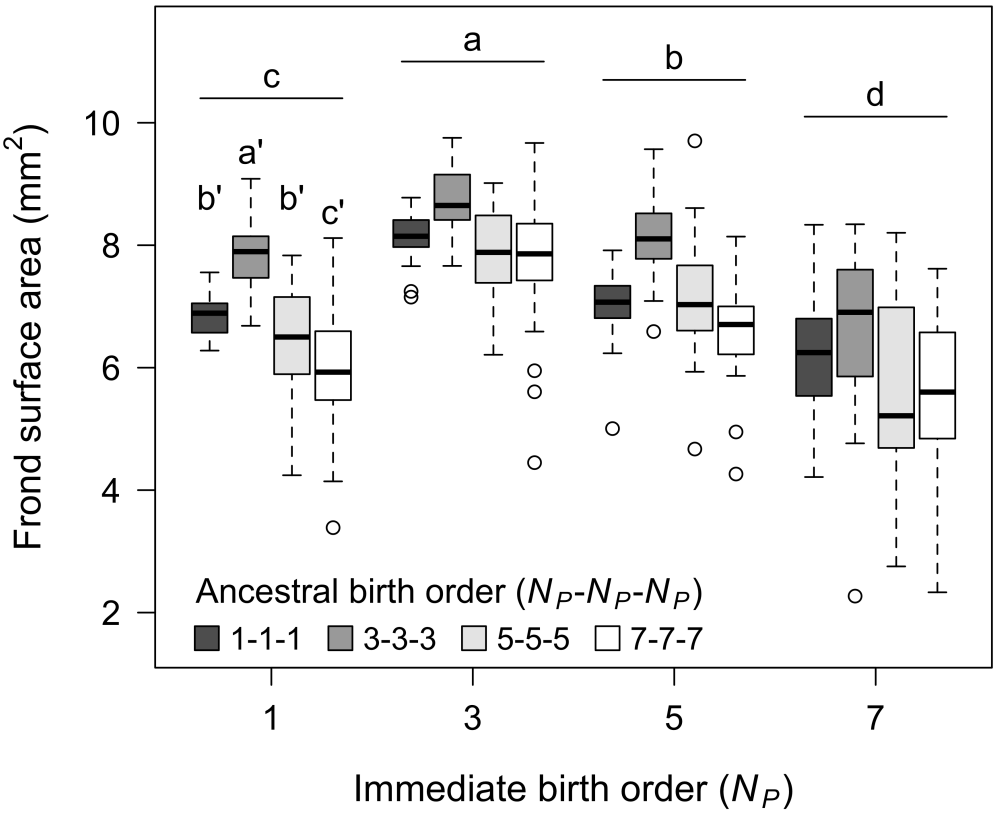

Figure 3-4. Frond surface area by immediate and ancestral birth order. Letters above the boxplots indicate significant differences among birth orders based on Tukey's post-hoc tests. For graphical clarity, post-hoc differences among ancestral birth orders are depicted only for the first level of immediate birth order, but actually apply to ancestral birth order independent of immediate birth order (as I did not model an interaction). Boxes depict the median and first and third quartiles, and whiskers extend to the lowest and highest data points within 1.5 times the interquartile-range of the first and third quartile, respectively.

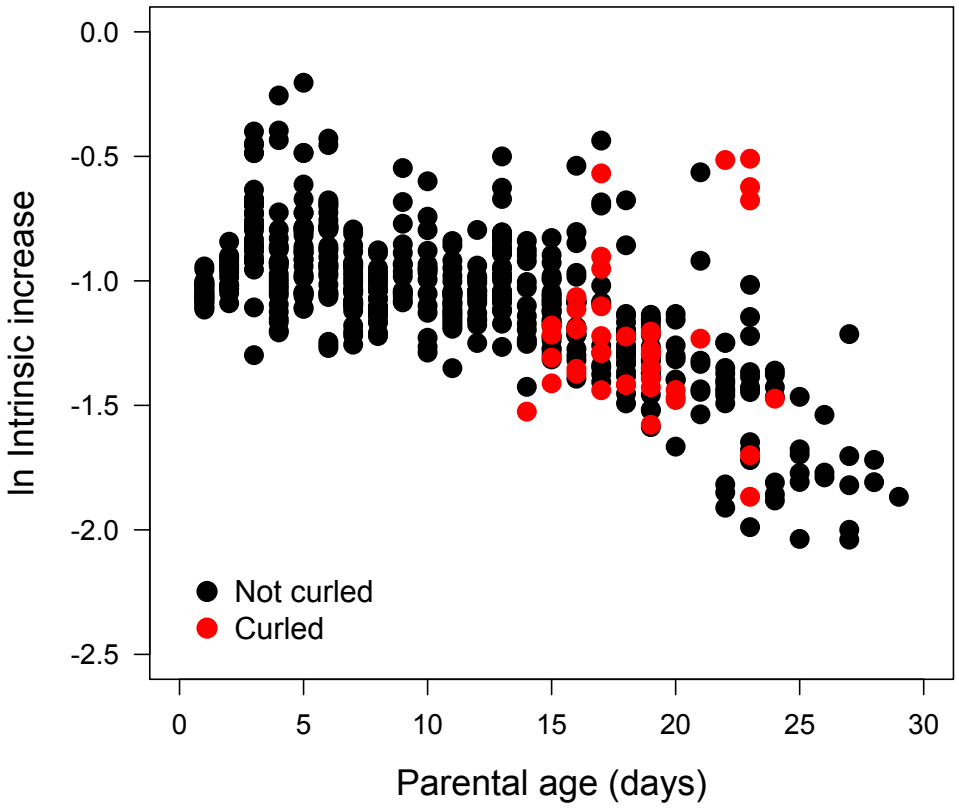

Figure 3-5. ln-Transformed individual intrinsic rates of increase versus parental age for curled (red) and non-curled (black) fronds. Data are from Chapter 2, and represent all 542 of the offspring detached from 41 parental fronds (a different genetic strain than was used in the current Chapter). The 41 parental fronds all had a birth-order genealogy of N_P-N_P-N_P-N_P = 1-1-1-1.

CHAPTER 4: PARENTAL AGE EFFECTS AND THE FORCE OF NATURAL SELECTION ON AGE-SPECIFIC VITAL RATES

Abstract

Theory on the evolution of senescence is largely based on models relating population growth rate to age-trajectories of survival and fecundity. Of particular interest is the 'sensitivity' of the population growth rate to small changes in age-specific vital rates (i.e. the age-specific 'force of natural selection'). W.D. Hamilton (1966) showed that this force of selection almost inevitably declines with increasing age – an observation key to understanding why senescence would ever evolve. In Hamilton's work, and most subsequent theory on the evolution of senescence, it is implicitly assumed that all offspring are of equal quality (i.e. senescence is defined only with respect to age-trajectories of survival and fecundity). However, many organisms are subject to age-related declines in offspring quality (known as a parental age effect) – an aspect of senescence that, until recently, has received little theoretical attention. Here, I extend the classic age-structured population projection model to also account for parental age structure, and apply this model to data from Chapter 2 to understand how parental age effects in *L. minor* influence the force of natural selection on age-specific vital rates. In accordance with results from recent theoretical work, my results suggest that age-related declines in offspring quality tend to increase the rate of age-related decline in the force of natural selection.

Introduction

A great deal of theory on the evolution of senescence is based on models (primarily the Euler-Lotka equation) that relate the population finite rate of increase λ (or intrinsic rate of increase, $r = \ln \lambda$) to age-trajectories of two vital rates: survival and fecundity (e.g. Hamilton 1966, Abrams 1993, Pedersen 1995, Sozou and Seymour 2004, Baudisch 2005). Of particular interest to those studying the evolution of senescence is the 'sensitivity' of λ to a hypothetical mutation that affects a single, age-specific vital rate, where sensitivity is defined as the partial derivative of λ with respect to the vital rate in question. Hamilton (1966) showed that such sensitivities almost inevitably decline with increasing age, because, assuming any nonzero level of mortality, fewer and fewer individuals survive to increasingly advanced age classes. Intuitively, there is little value to a mutation that benefits a specific age class if very few individuals survive to reach that age class in the first place. Furthermore, even in the absence of mortality, senescence "will tend to creep in" (Hamilton 1966, p. 25), because mutations that benefit an early age class at the expense of an older age class (known as 'antagonistic pleiotropy'; Williams 1957) will generally increase λ. Recent results suggest that age-related declines in the sensitivity of λ (also called the 'force of selection') are perhaps not as inevitable as Hamilton first proposed (Vaupel et al. 2004, Baudisch 2005, Caswell and Salguero-Gómez 2013). Nonetheless, Hamilton's results were foundational to the field, and his general approach continues to guide evolutionary theory on senescence (reviewed in Rose et al. 2007).

In Hamilton's work, and most subsequent theory on the evolution of senescence (including theory not specifically based on the Euler-Lotka equation, e.g. Vaupel et al. 2004), there is an implicit, simplifying assumption that all offspring are of equal quality, so that fitness and the force of selection depend only on age-trajectories of survival and fecundity. Relaxing this assumption (i.e. investigating the impact of variation in offspring quality on the force of selection) is potentially important for two reasons. First, there is plenty of evidence that offspring quality does in fact decline with increasing parental age across a wide range of taxa (Priest et al. 2002, Descamps et al. 2008, Bouwhouis et al. 2010, Gillespie et al. 2013b, Barks and Laird 2015). Thus, the implicit assumption that all offspring are of equal quality does not always hold. Second, recent theoretical results suggest that, if offspring quality does in fact decline with increasing parental age, classic methods that ignore such declines may underestimate age-related declines in the force of natural selection (Pavard et al. 2007a,b, Pavard and Branger 2012, Gillespie et al. 2013a). Just as there is little value, for example, in a mutation that increases fecundity within an advanced age class that few individuals survive to, intuitively, there is relatively little value in a mutation that increases fecundity within an age class that inevitably produces offspring of low quality. This is not to say that parental age effects must themselves be inevitable, but simply that, where they do occur, parental age effects may influence the force of selection on age-specific rates of survival and fecundity.

To my knowledge, the above-cited works (Pavard et al. 2007a,b, Pavard and Branger 2012, Gillespie et al. 2013a) are the only studies so far to examine parental age effects in the context of evolutionary theory on senescence. Pavard et al. model a

scenario where increasing maternal age increases the probability that offspring become orphaned, where orphans have a reduced chance of surviving to maturity compared to non-orphans. When Pavard et al. applied this model to data from human populations, the maternal age effect resulted in an increased overall force of selection on maternal survival, and a steeper age-related decline in the force of selection on maternal fecundity, compared to what was expected in the absence of the maternal age effect (Pavard et al. 2007a,b, Pavard and Branger 2012). Gillespie et al. model a hypothetical population comprised of two age classes and two birth orders (i.e. first-born vs. second-born), and allow fecundity to vary between the birth orders. When second-born offspring had lower fecundity than first-borns, there was a relatively steeper decline in the force of selection than when the two birth orders had equal fecundity (Gillespie et al. 2013a).

My goal here is to extend the above-described models (particularly the models of Pavard et al.) to allow for an arbitrary number of parental age classes, and a broader range of parental age effects (i.e. allow both survival and fecundity to vary with parental age over all possible age classes, not just the juvenile period). I then apply this model to data from Chapter #2 to understand how parental age effects in *L. minor* influence the force of natural selection on age-specific vital rates. Note that the parental age effect modeled here is limited to a single generation (i.e. vital rates are not affected by grandparental age, great-grandparental age, etc.), as per results described in Chapter #3.

Methods

OVERVIEW

In previous chapters, I argued that offspring quality should be recognized as a component of fitness that may vary with age, just like survival and fecundity. Here I approach parental effects from a slightly different angle. Instead of looking at how a single, composite measure of offspring fitness changes with parental age, I examine parental-age-related variation in age-trajectories of the two 'classic' fitness components – survival and fecundity (Fig. 4-1). In my opinion, this latter approach more readily facilitates the incorporation of parental age effects into existing theory.

My modeling approach closely follows Pavard and Branger (2012). I first develop a population projection model structured both by age and parental age (denoted $\boldsymbol{A}^{\mathrm{PAR}}$), and then derive a reference projection model ($\boldsymbol{A}^{\mathrm{REF}}$) that lacks parental age structure, but otherwise has equivalent dynamical properties to $\boldsymbol{A}^{\mathrm{PAR}}$ (i.e. the two models share the same finite rate of increase and stable age distribution). From each model, I derive sensitivities of λ to age-specific vital rates, and then compare age-trajectories of these sensitivities between the two models. Based on the above-described arguments, and results from relevant previous studies (Pavard et al. 2007a,b, Pavard and Branger 2012, Gillespie et al. 2013a), I expect that age-specific sensitivities from $\boldsymbol{A}^{\mathrm{PAR}}$ will decline with age more rapidly than sensitivities from $\boldsymbol{A}^{\mathrm{REF}}$ (i.e. accounting for parental age effects, where they occur, will yield a relatively steeper age-related decline in the force of selection).

BACKGROUND: PROJECTION OF AN AGE-STRUCTURED POPULATION

A simple starting point for any demographic analysis is the life cycle graph, which depicts the possible transitions between age classes (and/or stage classes) within a population over one time interval (Fig. 4-2). In a simple, age-structured population, transitions reflect either survival to the next age class, or reproduction, which always produces individuals of the youngest age class. If we know the number of individuals (n) in each age class (i) at a given time (t), we can use the life cycle graph and corresponding transition rates to 'project' the population over time – i.e. to determine the number of individuals expected in a given age class at some point in the future. For example, based on the hypothetical life cycle in Fig. 4-2, the number of individuals expected in age class 3 at time t+1 is simply the number of individuals in age class 2 at time t multiplied by S_2, the probability of surviving from age class 2 to age class 3, or

$$n_{3,(t+1)} = n_{2,t}S_2.$$

Likewise, the number of individuals expected in age class 2 at time t+1 is

$$n_{2,(t+1)} = n_{1,t}S_1.$$

Because individuals in the youngest age class only arise from reproduction (not survival), the number of individuals expected in age class 1 at time t+1 is the sum of the number of individuals in each age class i at time t multiplied by the per-capita fecundity for that age class:

$$n_{1,(t+1)} = n_{1,t}F_1 + n_{2,t}F_2 + n_{3,t}F_3.$$

While the individual equations above are potentially useful in their own right, they can also be combined into a system of equations that can be analyzed using methods from linear algebra. This is the basis of a population projection model. For

example, we can combine the number of individuals in each age class at a given time ($n_{i,t}$) into a population vector $\boldsymbol{n}_t$, as in

$$\boldsymbol{n}_t = \begin{pmatrix} n_1 \\ n_2 \\ \vdots \\ n_\alpha \end{pmatrix}_t,$$

where α is the maximum attainable age class in the population of interest. The vector $\boldsymbol{n}_t$ can be projected over time according to

$$\boldsymbol{n}_{t+1} = \boldsymbol{A}\boldsymbol{n}_t,$$

where $\boldsymbol{A}$ is a Leslie matrix of dimension $\alpha \times \alpha$, with per-capita fecundities for each age class (1 through α) across the top row, survival probabilities for age classes 1 through α-1 on the subdiagonal, and all other elements set to zero:

$$\boldsymbol{A} = \begin{pmatrix} F_1 & F_2 & \cdots & \cdots & F_\alpha \\ S_1 & & & & \vdots \\ \vdots & S_2 & & & \vdots \\ \vdots & & \ddots & & \vdots \\ 0 & \cdots & \cdots & S_{\alpha-1} & 0 \end{pmatrix}.$$

In addition to its application for iterative projection, we can derive a number of long-term population trends directly from projection model $\boldsymbol{A}$, which apply irrespective of the initial state of population vector $\boldsymbol{n}_t$ (as long as certain assumptions are met, as detailed in Caswell 2001, pp. 79-92). For example, the rate of population increase at equilibrium (λ) is given by the dominant eigenvalue of $\boldsymbol{A}$, and the age distribution at equilibrium ($\boldsymbol{w}$) is the scaled right dominant eigenvector. We can also obtain sensitivities to selection from $\boldsymbol{A}$, which represent the change in λ expected to result from a corresponding change in a given matrix element (i.e. an age-specific vital rate). The sensitivity of λ to matrix element $a_{k,l}$ (where k and l are row and column indices, respectively) is given by

$$\frac{\partial \lambda}{\partial a_{k,l}} = \frac{\bar{v}_k w_l}{\langle \boldsymbol{w}, \boldsymbol{v} \rangle},$$

where $\boldsymbol{w}$ and $\boldsymbol{v}$ are the dominant right and left eigenvectors of $\boldsymbol{A}$, respectively, $\bar{v}_k$ is the complex conjugate of the kth element of $\boldsymbol{v}$, and w_l is the lth element of $\boldsymbol{w}$ (Caswell 2001, p. 209).

INCORPORATING PARENTAL AGE EFFECTS

My goal is to extend the classic age-structured projection model to also incorporate parental age structure. This general approach of incorporating multiple state variables into a projection model is routine – such models are referred to as 'age-stage', 'multistate', or 'metapopulation' models (e.g. Caswell 2012).

I begin by illustrating how we might extend the age-structured life cycle graph in Fig. 4-2 to a graph that accounts for both age and parental age structure (see example in Fig. 4-3). In this new, multistate life cycle, transition rates ($S_{i,j}$ and $F_{i,j}$) depend not just on age class (i), but also now on parental age class (j). Transitions based on survival increment the age class but do not affect the parental age class, which is fixed at birth. Fecundity, on the other hand, always produces individuals in the youngest age class, but the parental age class transitioned *to* via fecundity depends on the age class transitioned *from* (i.e. the parental age class of the offspring depends on the age class of the parent). In the example within Fig. 4-3, the number of parental age classes is equal to the number of age classes, such that a parent within age class i produces offspring with parental age class $j = i$. This correspondence between age classes and parental age classes is convenient, but not

necessarily required (either biologically or mathematically). We could, for example, imagine that offspring quality is unaffected by parental age until very late in a parent's life, at which point offspring quality declines. In this case, we might prefer to group some of the early age classes together into one parental age class, as in Fig. 4-4. If so, we must be careful to keep track of the mapping between age classes and parental age classes.

GENERALIZING TO α AGE CLASSES AND β PARENTAL AGE CLASSES

The population vector $\boldsymbol{n}_t^{\mathrm{PAR}}$ describes a population structured both by age and parental age. It can be written in terms of 'blocks' or sub-vectors, as in

$$\boldsymbol{n}_t^{\mathrm{PAR}} = \begin{pmatrix} \text{parental age class 1} \\ \hline \text{parental age class 2} \\ \hline \vdots \\ \hline \text{parental age class } \beta \end{pmatrix}_t = \begin{pmatrix} n_{1,1} \\ \vdots \\ \hline n_{\alpha,1} \\ \hline n_{1,2} \\ \vdots \\ \hline n_{\alpha,2} \\ \vdots \\ \hline n_{1,\beta} \\ \vdots \\ n_{\alpha,\beta} \end{pmatrix}_t,$$

where $n_{i,j,t}$ is the number of individuals in age class i and parental age class j at time t, α is the maximum attainable age class, and β the maximum attainable parental age class ($\beta \leq \alpha$). Here, I have chosen to block the population vector (and corresponding projection matrix) by parental age class as opposed to age class, but this decision is arbitrary and simply requires consistency. Recall that, if β is strictly less than α, then at least one parental age class contains more than one age class, in which case we must track the mapping between age classes and parental age classes. For this

purpose, I define a set for each parental age class j, map_j, whose elements are the age class(es) associated with the given parental age class. For example, in Fig. 4-4, $map_1 = \{1, 2\}$ and $map_2 = \{3\}$.

The population vector $\boldsymbol{n}_t^{\mathrm{PAR}}$ can be projected through time according to

$$\boldsymbol{n}_{t+1}^{\mathrm{PAR}} = \boldsymbol{A}^{\mathrm{PAR}}\boldsymbol{n}_t^{\mathrm{PAR}},$$

where $\boldsymbol{A}^{\mathrm{PAR}}$ is a projection matrix reflecting the same age and parental age structure as $\boldsymbol{n}_t^{\mathrm{PAR}}$. Specifically, $\boldsymbol{A}^{\mathrm{PAR}}$ is composed of blocks or submatrices denoted $\boldsymbol{P}_{x,y}$ and $\boldsymbol{M}_{x,y}$, which give transition probabilities (based on survival and fecundity, respectively) to parental age class x from parental age class y, where x and y are integers from 1 through β:

$$\boldsymbol{A}^{\mathrm{PAR}} = \begin{pmatrix} \boldsymbol{P}_{1,1} & \mathbf{0} & \cdots & \mathbf{0} \\ \mathbf{0} & \boldsymbol{P}_{2,2} & \cdots & \mathbf{0} \\ \vdots & \vdots & \ddots & \vdots \\ \mathbf{0} & \mathbf{0} & \cdots & \boldsymbol{P}_{\beta,\beta} \end{pmatrix} + \begin{pmatrix} \boldsymbol{M}_{1,1} & \boldsymbol{M}_{1,2} & \cdots & \boldsymbol{M}_{1,\beta} \\ \boldsymbol{M}_{2,1} & \boldsymbol{M}_{2,2} & \cdots & \boldsymbol{M}_{2,\beta} \\ \vdots & \vdots & \ddots & \vdots \\ \boldsymbol{M}_{\beta,1} & \boldsymbol{M}_{\beta,2} & \cdots & \boldsymbol{M}_{\beta,\beta} \end{pmatrix},$$

where $\mathbf{0}$ represents a $\alpha \times \alpha$ matrix with all elements set to zero. Recall that transitions based on survival do not alter the parental age class (which is fixed at birth), so all submatrices $\boldsymbol{P}_{x,y}$ for which $x \neq y$ are null. For transitions based on fecundity, the parental age class transitioned to (x) depends on the age class transitioned from, but is independent of the parental age class transitioned from (y), so we must consider submatrices $\boldsymbol{M}_{x,y}$ over all combinations of x and y.

Submatrices $\boldsymbol{P}_{x,y}$ and $\boldsymbol{M}_{x,y}$ (both of dimension $\alpha \times \alpha$) represent the basic building blocks of a Leslie matrix, with matrices $\boldsymbol{P}_{x,y}$ giving survival probabilities for each age class on the subdiagonal, and matrices $\boldsymbol{M}_{x,y}$ giving per-capita fecundities for each age class across the top row. Specifically,

$$\boldsymbol{P}_{\boldsymbol{x},\boldsymbol{y}(\boldsymbol{x}=\boldsymbol{y})} = \begin{pmatrix} 0 & \cdots & \cdots & \cdots & 0 \\ S_{1,j} & & & & \vdots \\ \vdots & S_{2,j} & & & \vdots \\ \vdots & & \ddots & & \vdots \\ 0 & \cdots & \cdots & S_{(\alpha-1),j} & 0 \end{pmatrix},$$

where $S_{i,j}$ is the probability of surviving through age class *i* for an individual with parental age class *j* (*j* = *x* = *y* because survival does not alter the parental age class). Because I wish to allow for the possibility of fewer parental age classes than age classes (β may be strictly less than α), the construction of submatrices $\boldsymbol{M}_{x,y}$ requires an additional step that maps age classes to parental age classes. Specifically, I define

$$\boldsymbol{M}_{\boldsymbol{x},\boldsymbol{y}} = \begin{pmatrix} \delta_{1,j,x} & \delta_{2,j,x} & \cdots & \delta_{\alpha,j,x} \\ 0 & 0 & \cdots & 0 \\ \vdots & \vdots & \ddots & \vdots \\ 0 & \cdots & \cdots & 0 \end{pmatrix},$$

where $\delta_{i,j,x}$ represents the probability of transition (based on fecundity) from an individual in age class *i* and parental age class *j* (*j* = *y*, the parental age class transitioned from), to an individual in age class 1 (fecundity only ever produces individuals of the youngest age class) and parental age class *x* (the parental age class transitioned to). The values $\delta_{i,j,x}$ within this matrix are null except where $i \in map_x$ (i.e. where the age class of the individual transitioned from falls within the parental age class being transitioned to). Specifically,

$$\delta_{i,j,x} = \begin{cases} F_{i,j}, & i \in map_x \\ 0, & \text{else} \end{cases},$$

where $F_{i,j}$ is the per-capita fecundity for an individual in age class *i* and parental age class *j* (*j* = *y*, the parental age class transitioned from). To clarify, the age class of the parent does not necessarily become the parental age class of the offspring (unless $\beta = \alpha$, in which case age classes and parental age classes correspond exactly). Rather,

and more generally, the parental age class that the parent's age falls within becomes the parental age class of the offspring.

ANALYSIS OF $\boldsymbol{A}^{\mathrm{PAR}}$

Despite its unique construction from submatrices reflecting different parental age classes, $\boldsymbol{A}^{\mathrm{PAR}}$ is fundamentally a multistate projection model that can be analyzed using standard methods (e.g. Caswell 2001). The population growth rate at equilibrium (λ^{PAR}), stable age-by-parental-age distribution ($\boldsymbol{w}^{\mathrm{PAR}}$), and sensitivities of λ^{PAR} to age-by-parental-age-specific vital rates ($S_{i,j}, F_{i,j}$) are derived in the same way for $\boldsymbol{A}^{\mathrm{PAR}}$ as previously described for a standard Leslie matrix. However, the stable distribution and sensitivities derived from $\boldsymbol{A}^{\mathrm{PAR}}$ reflect age-by-parental-age classes, whereas, to facilitate comparison with a reference model lacking parental age structure, I wish to derive the stable *age* distribution ($\tilde{\boldsymbol{w}}^{\mathrm{PAR}}$) and sensitivities of λ^{PAR} to *age*-specific vital rates ($\tilde{S}_i, \tilde{F}_i$).

Deriving the stable age distribution from the stable age-by-parental-age distribution is straightforward. The relative abundance of age class *i* at equilibrium ($\tilde{w}_i^{\mathrm{PAR}}$) is simply the sum of the relative age-by-parental-age abundances for that age class over all parental age classes, or

$$\tilde{w}_i^{\mathrm{PAR}} = \sum_{j=1}^{\beta} w_{i,j}^{\mathrm{PAR}}.$$

Coincidently, the sensitivity of λ^{PAR} to a given age-specific vital rate ($\tilde{S}_i$ or $\tilde{F}_i$) is determined in a similar manner, by summing the relevant sensitivities for a given age class over all parental age classes, as in

$$\frac{\partial \lambda^{\mathrm{PAR}}}{\partial \tilde{S}_i} = \sum_{j=1}^{\beta} \frac{\partial \lambda^{\mathrm{PAR}}}{\partial S_{i,j}}.$$

This summation is appropriate because, in general, the sensitivity of λ to a factor z that *equally affects* matrix elements $a_{k,l}$ to $a_{m,n}$ is the sum of the of the sensitivities of λ to each individually-perturbed element (Caswell 2001, p. 219), or

$$\frac{\partial \lambda}{\partial z} = \sum_{k,l}^{m,n} \frac{\partial \lambda}{\partial a_{i,j}}.$$

Age-specific sensitivities derived from $\boldsymbol{A}^{\mathrm{PAR}}$ in this manner can therefore be understood as the change in λ^{PAR} expected to result from a hypothetical mutation that affects an age-specific vital rate for all members of the given age class, regardless of their parental age class.

REFERENCE MODEL

To understand the evolutionary consequences of parental age effects, I compare age-specific sensitivities derived from $\boldsymbol{A}^{\mathrm{PAR}}$ to those derived from a reference model ($\boldsymbol{A}^{\mathrm{REF}}$) that lacks parental age structure, but otherwise has equivalent dynamical properties ($\lambda^{\mathrm{REF}} = \lambda^{\mathrm{PAR}}$, $\boldsymbol{w}^{\mathrm{REF}} = \widetilde{\boldsymbol{w}}^{\mathrm{PAR}}$). The reference model is a standard, age-classified Leslie matrix (dimensions $\alpha \times \alpha$) with survival probabilities on the subdiagonal and per-capita fecundities across the top row:

$$\boldsymbol{A}^{\mathrm{REF}} = \begin{pmatrix} \bar{F}_1 & \bar{F}_2 & \cdots & \cdots & \bar{F}_\alpha \\ \bar{S}_1 & & & & \vdots \\ \vdots & \bar{S}_2 & & & \vdots \\ \vdots & & \ddots & & \vdots \\ 0 & \cdots & \cdots & \bar{S}_{\alpha-1} & 0 \end{pmatrix}.$$

Transition rates in the reference model ($\bar{S}_i$ and $\bar{F}_i$) are based on mean, age-specific transition rates expected at equilibrium under $\boldsymbol{A}^{\mathrm{PAR}}$. With respect to fecundity, this

entails a weighted summation of age-by-parental-age-specific fecundities ($F_{i,j}$) across all parental age classes, with each $F_{i,j}$ weighted by the relative proportion of parental age class *j* comprising age class *i* at the stable distribution of $\boldsymbol{A}^{\mathrm{PAR}}$, or

$$\bar{F}_i = \sum_{j=1}^{\beta} F_{i,j} \frac{w_{i,j}^{\mathrm{PAR}}}{\widetilde{w}_i^{\mathrm{PAR}}}.$$

Age-specific survival probabilities expected at equilibrium can be determined similarly, as in

$$\bar{S}_i = \sum_{j=1}^{\beta} S_{i,j} \frac{w_{i,j}^{\mathrm{PAR}}}{\widetilde{w}_i^{\mathrm{PAR}}},$$

or, can equivalently be derived directly from the stable distribution (Caswell 2001, pp. 154-155) of $\boldsymbol{A}^{\mathrm{PAR}}$ according to

$$\bar{S}_i = \lambda^{\mathrm{PAR}} \frac{\widetilde{w}_{i+1}^{\mathrm{PAR}}}{\widetilde{w}_i^{\mathrm{PAR}}}.$$

Once $\boldsymbol{A}^{\mathrm{REF}}$ has been populated with the transition rates described above, I use standard techniques (described in the *Background* section) to determine the sensitivity of λ^{REF} to $\bar{S}_i$ and $\bar{F}_i$. I then compare these sensitivities to their counterparts from projection model $\boldsymbol{A}^{\mathrm{PAR}}$ – the sensitivity of λ^{PAR} to $\tilde{S}_i$ and $\tilde{F}_i$, respectively.

PARAMETER VALUE SPECIFICATION FROM EMPIRICAL DATA

The values in $\boldsymbol{A}^{\mathrm{PAR}}$ that need to be specified include α and β, the maximum attainable age and parental age classes, sets map_j, which give the mapping between age and parental age classes, and age-by-parental-age-specific transition rates $S_{i,j}$ and $F_{i,j}$. There are potentially many reasonable ways to specify each of these

parameters, and therefore many 'researcher degrees of freedom' (*sensu* Simmons et al. 2011). For instance, demographers rarely consider age classes up to the *actual* maximum attainable age class in a population, because low sample sizes in advanced age classes result in a high degree of parameter value uncertainty. Thus, I might reasonably settle on a value for α that is lower than the empirical maximum. For similar reasons (i.e. sample-size-related considerations), there may be more than one reasonable way to select the number of parental age classes (β), and the mapping between age and parental age classes (sets map_j). Furthermore, regardless of the specification of α, β, and map_j, there are many reasonable ways to estimate transition rates (Caswell 2001, Ch. 6). For instance, model transition rates could be based on raw empirical values, non-parametrically-smoothed empirical values, or values derived from a parametric model fit to empirical data. These latter two categories come with a host of additional choices, such as type of model, model constraints, etc. Given the many degrees of freedom, my approach to parameter specification is simply to try a number of specification combinations, and report the results for each.

The dataset that I used to estimate parameters for $\boldsymbol{A}^{\mathrm{PAR}}$ is described in *Phase two* of Chapter #2. In that study, I tracked all of the offspring (N = 542 focal fronds) detached from 41 parental fronds. The maximum age attained by a focal frond was 44 days, and the maximum parental age was 29 days (Fig. 4-5) (the disparity between maximum age and parental age likely just reflects the different sample sizes, 542 vs. 41). Fewer than 5% of the 542 focal fronds survived beyond the age of 30 days, so, in specifying $\boldsymbol{A}^{\mathrm{PAR}}$, I chose not to consider values of α greater than 30.

I specified $\boldsymbol{A}^{\mathrm{PAR}}$ according to three separate specification regimes (outlined in Table 4-1, depicted in Fig. 4-6). In the first regime (Specification #1), I used a small number of parental age classes ($\beta = 4$) so as to attain a relatively large sample size within each, and specified transition rates based on raw, empirically-observed values. In Specification #2, I considered twice as many parental age classes ($\beta = 8$), and used non-parametric smoothing to estimate transition rates. Here, smoothing was attained via generalized additive models (GAMs) relating survival (binomial error) or number of offspring released (Gaussian error) to age. I fit models separately to each parental age class using the *gam* function in the R package MGCV. For Specifications #1 and #2, after selecting values for α and β, I specified sets map_j by minimizing the among-parental-age-class variance in sample size (in each case, the number of possible combinations was small enough to facilitate an exhaustive search for minimum variance). For Specification #3, I set $\alpha = \beta$ so that there was a one to one correspondence between age classes and parental age classes. Survival rates in Specification #3 were based on raw, empirical values determined for the total population (all parental age classes combined; i.e. $S_{i,1} = S_{i,2} = \ldots = S_{i,\beta}$). I elected to use population-average survival rates in one specification because there was no statistical evidence for an effect of parental age on survival (Cox proportional hazards regression, coefficient for parental age = 1.002, 95% CI [0.99, 1.02]). Fecundities in Specification #3 were estimated via a single GAM (Gaussian error) that included a tensor product interaction between age and parental age (roughly analogous to an interaction term in a general linear model).

Results

Regardless of the specification regime, age-specific sensitivities derived from $\boldsymbol{A}^{\mathrm{PAR}}$ (i.e. the sensitivity of λ^{PAR} to $\tilde{S}_i$ and $\tilde{F}_i$) were greater among the youngest age classes and lower among the oldest age classes than sensitivities derived from $\boldsymbol{A}^{\mathrm{REF}}$ (i.e. the sensitivity of λ^{REF} to $\bar{S}_i$ and $\bar{F}_i$) (Figs. 4-7 and 4-8). Overall, sensitivities derived from $\boldsymbol{A}^{\mathrm{PAR}}$ declined with age more rapidly than those from $\boldsymbol{A}^{\mathrm{REF}}$. For all specification regimes, sensitivities derived from $\boldsymbol{A}^{\mathrm{PAR}}$ declined monotonically both with increasing age and parental age (Fig. 4-9). That is, for a given parental age class, sensitivities consistently declined with increasing age, and similarly, for a given age class, sensitivities consistently declined with increasing parental age.

Discussion

As predicted, accounting for parental age effects (i.e. a decline in offspring quality with increasing parental age), where they occurred, led to a relatively steeper age-related decline in the force of selection on survival and fecundity, compared to what was expected if parental age effects were ignored. While this result is perhaps intuitive (e.g. Kern et al. 2001, my arguments in the Introduction), and corresponds with results from previous studies (Pavard et al. 2007a,b, Pavard and Branger 2012, Gillespie et al. 2013a), to my knowledge, there are no formal analytical results that explain why (or under what conditions) parental age effects should lead to a steeper age-related decline in the force of selection. Because a great deal of evolutionary theory on senescence is fundamentally based on age-related

declines in the force of selection, further incorporating parental age effects into theory on senescence is an important challenge.

In the absence of analytical results, further understanding of how parental age effects influence the force of selection could be obtained by applying the projection model and general framework used here to other empirical datasets. For those so inclined, one special consideration to note is that projection models of this type may be particularly prone to 'reducibility', which can occur when there are dead ends or disconnects within the life cycle (i.e. stages cut off from the rest, or from which no subsequent transitions can occur) (Caswell 2001, pp. 79-92). The potential problem with reducible projection matrices is that they may be non-ergodic, in which case long-term population dynamics (e.g. λ, $\boldsymbol{w}$) depend on the initial state of the population vector $\boldsymbol{n}_t$. In specifying parameters for a projection model structured both by age and parental age, to avoid non-ergodicity, one should ensure that, within each parental age class, there is a nonzero probability of transitioning to (from) each age class up to α (α-1) (though this does not in itself guarantee irreducibility). One way to facilitate this might be to specify α based on the parental age class for which there is the lowest survival, or for which reproduction ceases earliest. Stott et al. (2010) note that a surprising number of published projection models exhibit non-ergodicity, not because the modeled life cycle was inherently irreducible, but rather because not all transition rates were specified with nonzero values. They helpfully provide R scripts to test any population projection matrix both for reducibility and ergodicity (Stott et al. 2010).

In the current study, although age-specific sensitivities from $\boldsymbol{A}^{\mathrm{PAR}}$ declined more rapidly with age than those from $\boldsymbol{A}^{\mathrm{REF}}$, the absolute difference in age-specific sensitivities between $\boldsymbol{A}^{\mathrm{PAR}}$ and $\boldsymbol{A}^{\mathrm{REF}}$ was small compared to the range in sensitivities across all age classes (this range spanned almost 8 orders of magnitude; see Fig. 4-7). Given this relatively small difference in sensitivities, and the fact that the data modeled here were collected under optimal, laboratory conditions, my results do not necessarily provide evidence that the parental age effect in *L. minor* is evolutionarily significant. As with all models, mine is a simplification of reality. In particular, I have not considered density-dependence (Caswell 2001, Ch. 16), genetic covariation among age-specific vital rates (Charlesworth 1990), or stochasticity (either environmental or demographic; Tuljapurkar et al. 2009), each of which may influence demographic or evolutionary dynamics. Nonetheless, my results correspond qualitatively with findings from previous studies (Pavard et al. 2007a,b, Pavard and Branger 2012, Gillespie et al. 2013a). Together, these results suggest that, at least under some conditions, parental age effects will modify age-trajectories of selection. Because parental age effects are known to occur in a wide range of taxa (e.g. Priest et al. 2002, Descamps et al. 2008, Bouwhouis et al. 2010, Gillespie et al. 2013b, Barks and Laird 2015), further incorporation of such effects into evolutionary theory on senescence is warranted.

Table 4-1. Outline of the three parameter specification regimes for $\mathbf{A}^{\mathrm{PAR}}$.

Parameter	Spec. #1	Spec. #2	Spec. #3
α	30	30	25
β	4	8	25
map_j j{age classes i} (sample size)	1{1-5} (138) 2{6-11} (138) 3{12-17} (139) 4{18-29} (127)	1{1-3} (80) 2{4-5} (58) 3{6-7} (60) 4{8-11} (78) 5{12-14} (63) 6{15-17} (76) 7{18-20} (63) 8{21-29} (64)	1:1 mapping (sample sizes for each parental age class depicted in Fig. 4-5)
$S_{i,j}$	Raw empirical	Smoothed via GAM (binomial error)	Raw empirical (population average)
$F_{i,j}$	Raw empirical	Smoothed via GAM (Gaussian error)	Smoothed via GAM (Gaussian error) with tensor product interaction between age and parental age

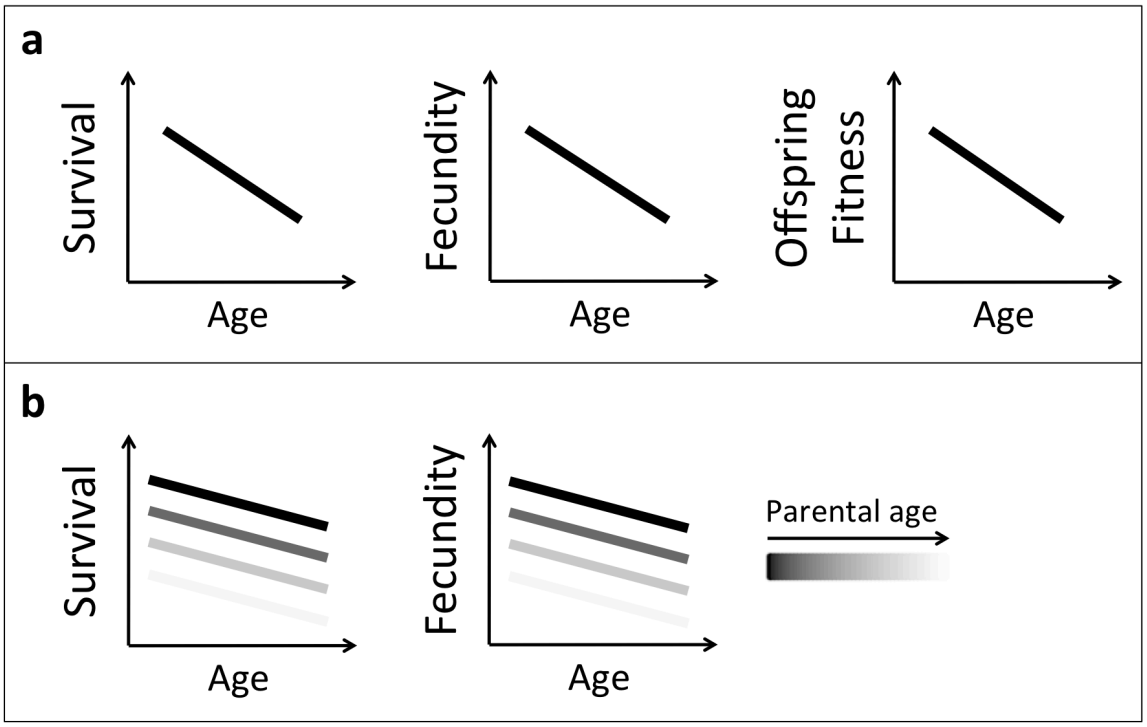

Figure 4-1. Two conceptual representations of parental age effects. Panel **a** depicts age-related variation in three fitness components: survival, fecundity, and offspring fitness. Panel **b** depicts age-related *and* parental-age-related variation in the two 'classic' fitness components: survival and fecundity.

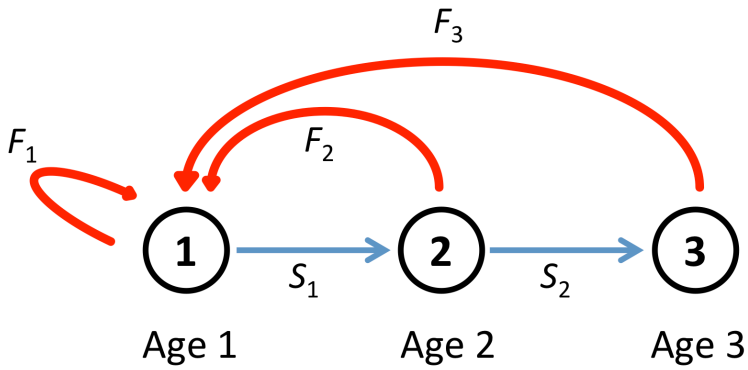

Figure 4-2. Life cycle graph for a hypothetical organism with three age classes. Blue arrows represent transitions based on survival (the transition rate for age class *i* is given by S_i), whereas red arrows reflect fecundity (transition rate F_i).

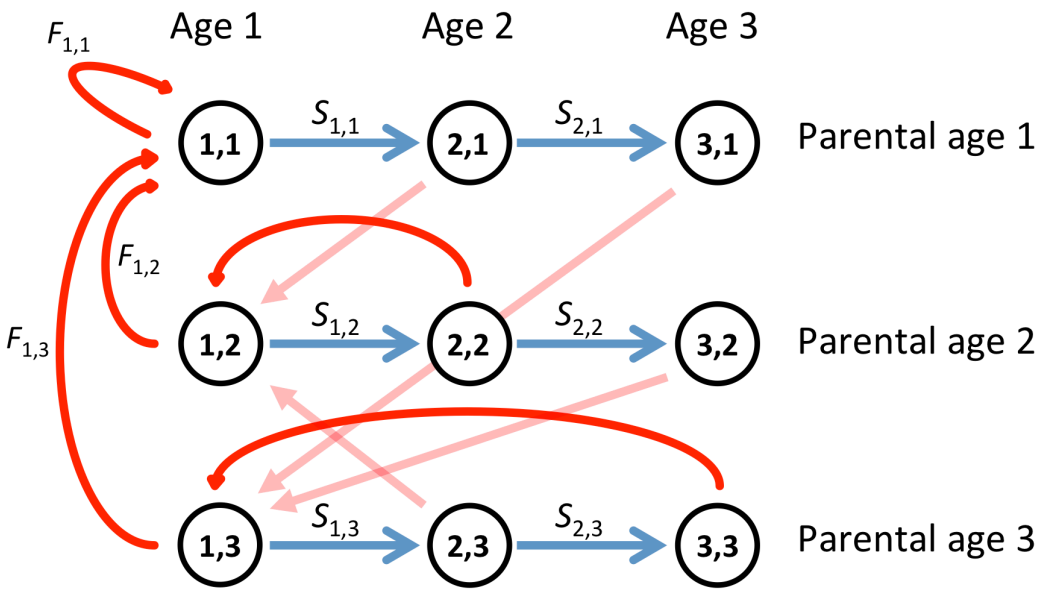

Figure 4-3. Life cycle graph for a hypothetical organism with three age classes and three parental age classes. Blue arrows represent transitions based on survival (the transition rate for age class *i* and parental age class *j* is given by $S_{i,j}$), whereas red arrows reflect fecundity (transition rate given by $F_{i,j}$). Note that some fecundity labels are omitted from the figure to limit clutter.

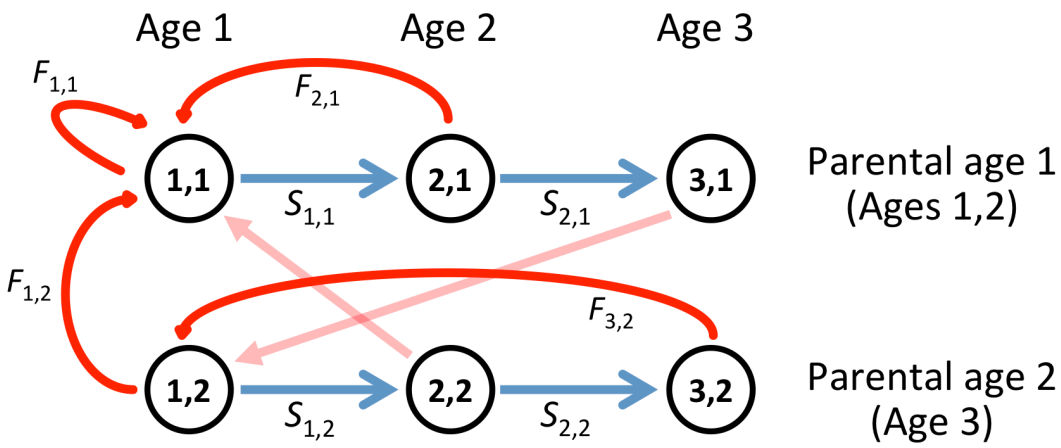

Figure 4-4. Life cycle graph for a hypothetical organism with three age classes and two parental age classes. Blue arrows represent transitions based on survival (the transition rate for age class *i* and parental age class *j* is given by $S_{i,j}$), whereas red arrows reflect fecundity (transition rate given by $F_{i,j}$). Note that some fecundity labels are omitted from the figure to limit clutter.

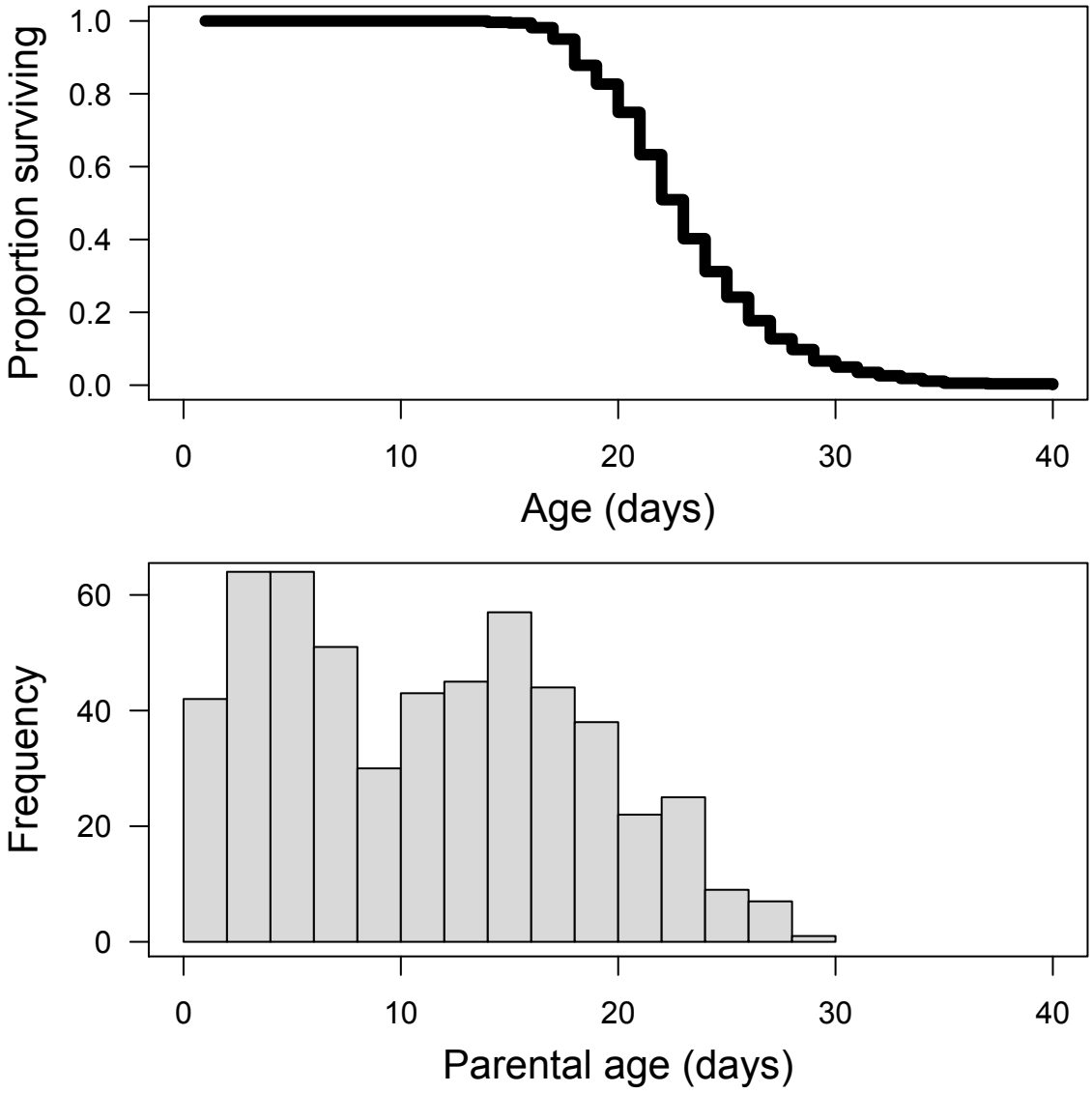

Figure 4-5. Survivorship (top) and frequency of parental age classes (bottom) among the 542 focal fronds in *Phase two* of Chapter 2.

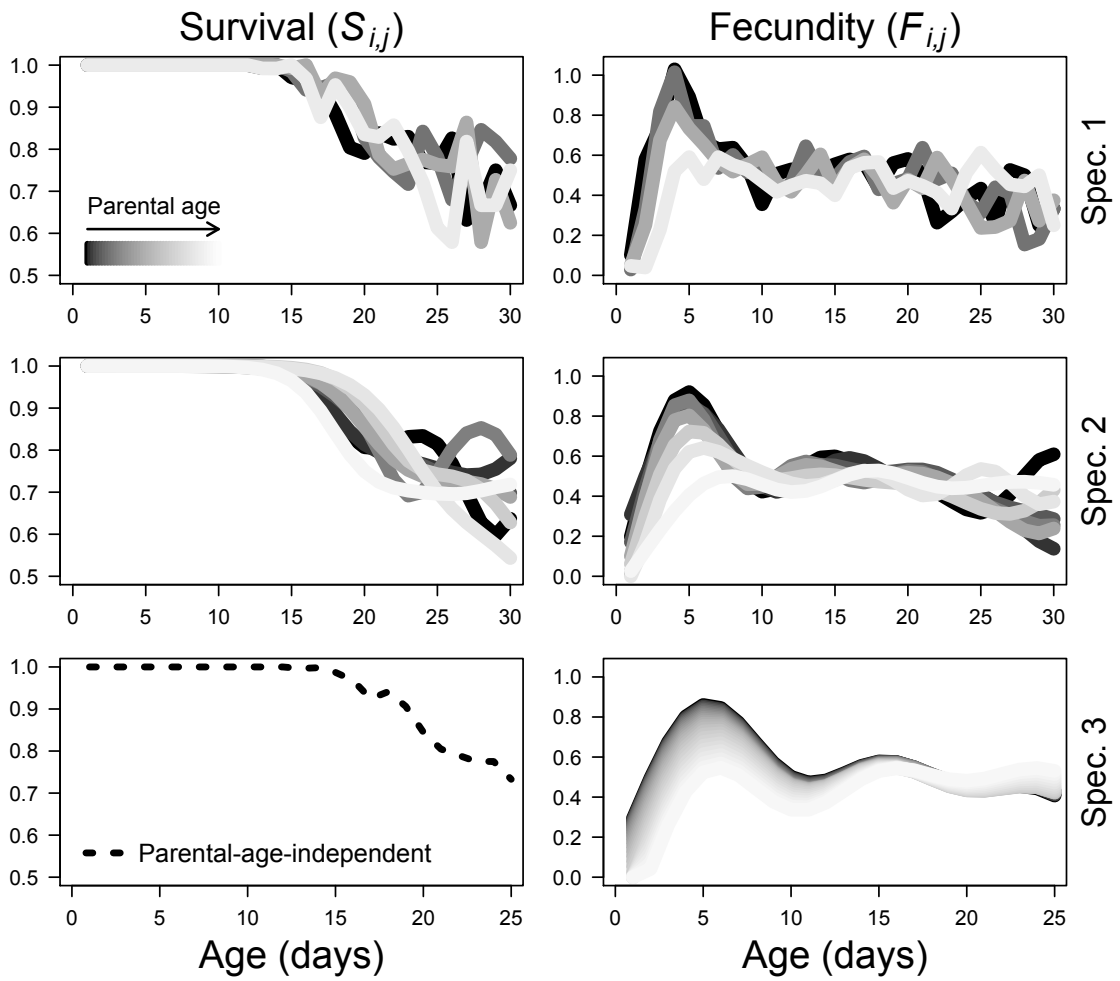

Figure 4-6. Transition rates ($S_{i,j}$ and $F_{i,j}$) used to specify $\boldsymbol{A}^{\mathrm{PAR}}$ under three separate specification regimes (details in Table 4-1).

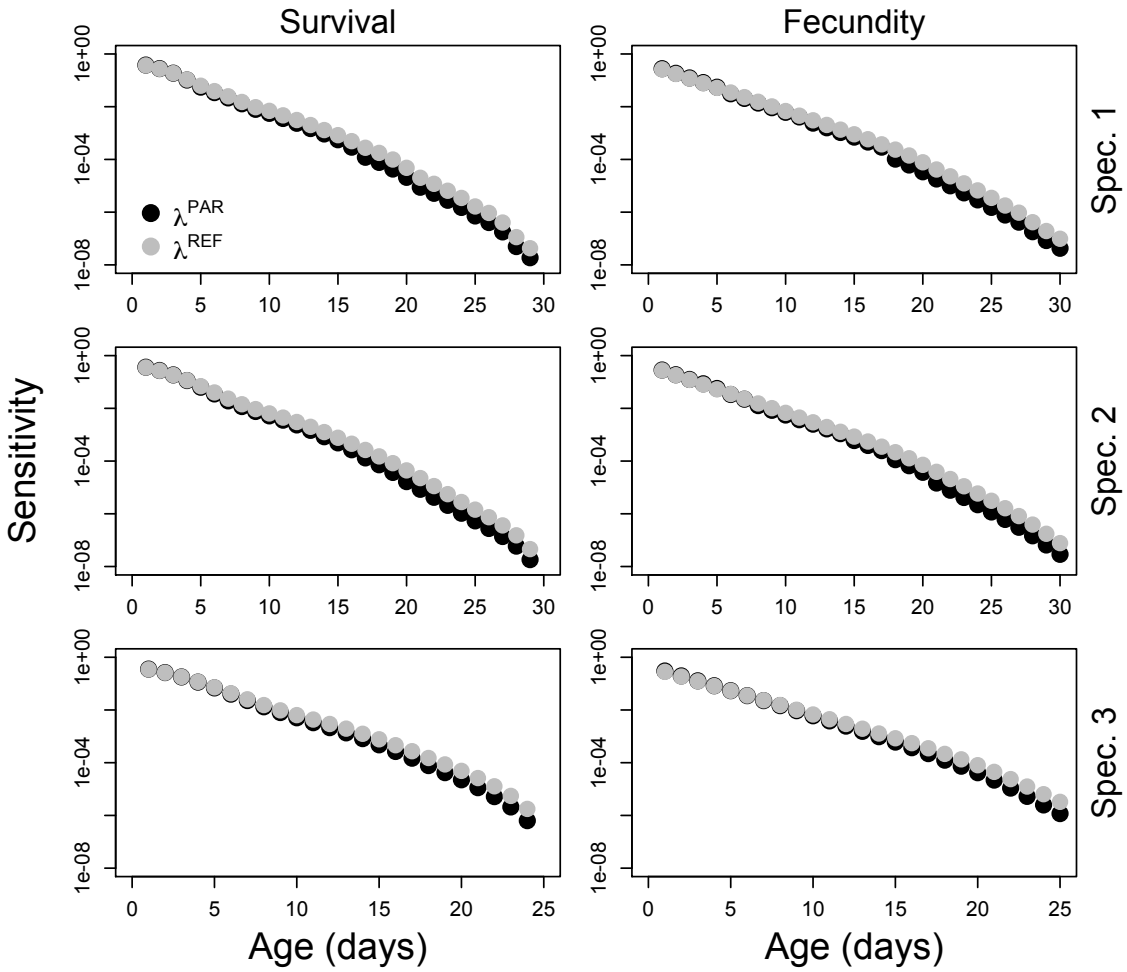

Figure 4-7. Sensitivity of λ^{PAR} (black circles) and λ^{REF} (grey circles) to age-specific transition rates, under three separate specification regimes (details in Table 4-1).

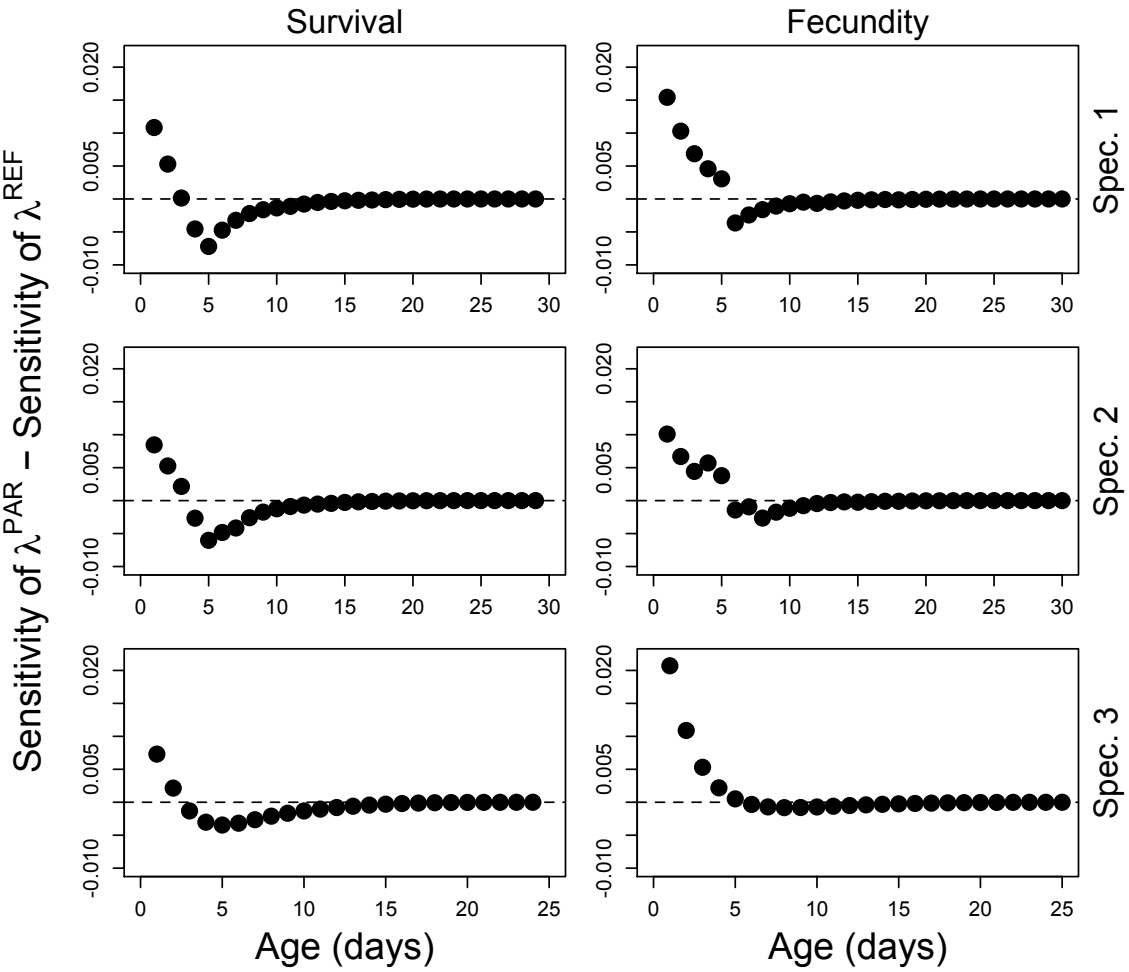

Figure 4-8. Difference in sensitivities of λ (sensitivity of λ^{PAR} – sensitivity of λ^{REF}) to age-specific transition rates, under three separate specification regimes (details in Table 4-1).

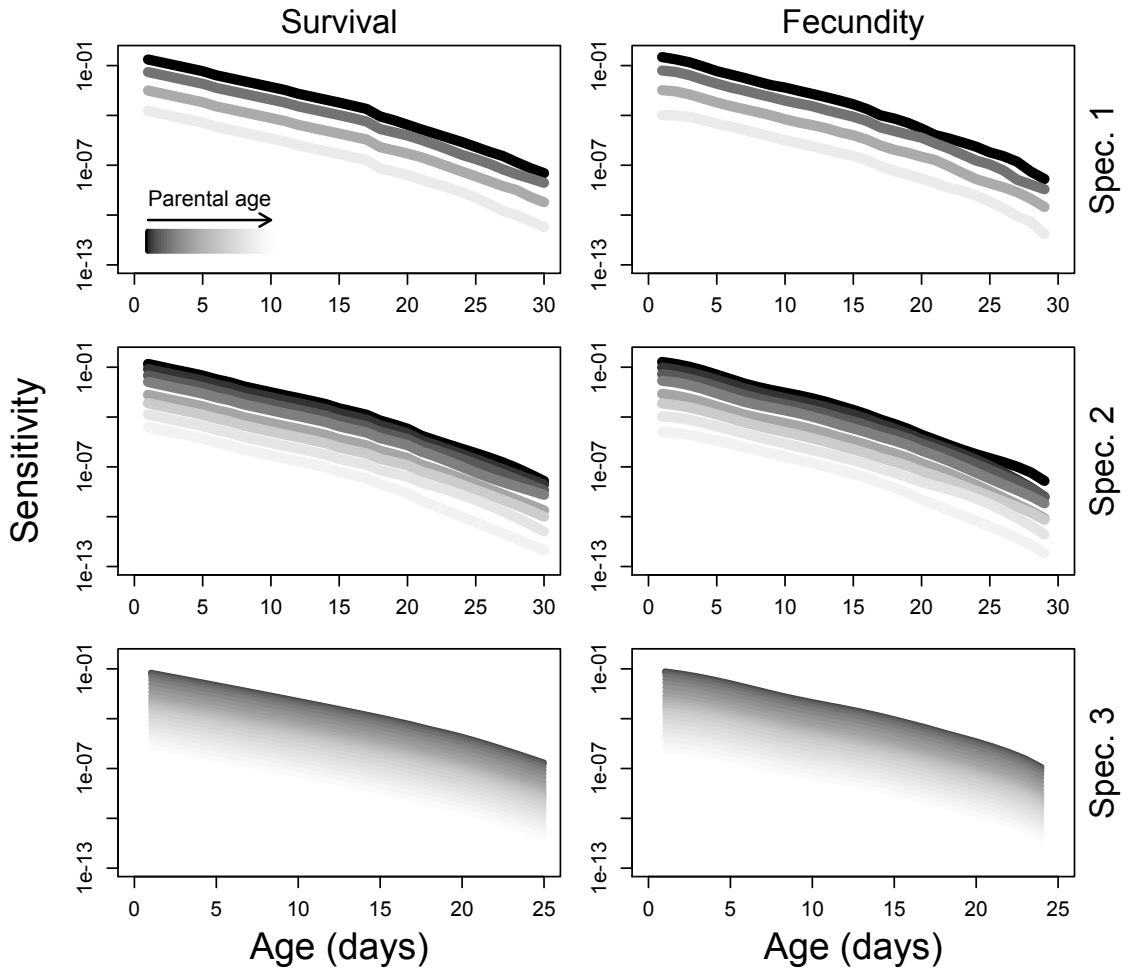

Figure 4-9. Sensitivity of λ^{PAR} to age-by-parental-age specific transition rates under three separate specification regimes (details in Table 4-1).

CHAPTER 5: AMONG-POPULATION CONSISTENCY IN LIFE EXPECTANCY AND RATE OF SENESCENCE IN COMMON DUCKWEED (*LEMNA MINOR*)

Abstract

Both within and among species, there exists a great deal of variation in life expectancy and rates of actuarial senescence (i.e. rates of increase in morality with age), and much of this variation remains to be explained. Because of the difficulties associated with phylogenetic non-independence in comparisons across taxa, intraspecific studies are particularly well suited for examining extrinsic forces underlying variation in rates of senescence. Whereas there are many such studies in animals, little is known about within-species variation in rates of senescence in plants. Here I describe a common garden study examining variation in life history traits (focusing on life expectancy and rate of senescence) across 28 strains of the aquatic plant *Lemna minor*, derived from 23 wetlands in Alberta, Canada. I observed among-strain variation (and within-strain consistency) in plant size and number of offspring, but little variation in life expectancy or rate of senescence. I found mixed evidence for a negative relationship between plant size in the common garden and nutrient concentrations at the sites of origin. Recent research suggests that angiosperms overwhelmingly do not exhibit senescence (in contrast to most other taxonomic groups), but still display extensive among-species variability in age-trajectories of mortality. My results suggest that age-trajectories of mortality are highly conserved within one particular angiosperm species, despite among-strain variability in other life history traits.

Introduction

Both within and among species, there exists a great deal of variation in life expectancy. For example, among vertebrate animals, maximum lifespan varies from just a few months in the Labord's chameleon (*Furcifer labordi*) to over 200 years in the rougheye rockfish (*Sebastes aleutianus*) (de Magalhães and Costa 2009). An even wider range of variation occurs among vascular plants, with life expectancies ranging from a few months to thousands of years (Noodén 1988). Variation in life expectancy *within* species is also common (e.g. Bronikowski and Arnold 1999, Reznick et al. 2004, Carlson et al. 2007, Terzibasi Tozzini et al. 2013).

Life expectancy is a composite measure reflecting age-specific rates of mortality integrated over the entire life history. Because rates of mortality tend to change with age (typically increasing with age, often in a curvilinear manner; Jones et al. 2014), differences in life expectancy among populations or taxa can arise in many different ways. Researchers often partition life expectancy into at least two broad components (e.g. Pletcher et al. 2000): age-independent mortality, and the rate of change in mortality with age (also called the rate of 'actuarial senescence'). Variation in this latter component has been the subject of a great deal of research because evolutionary theory predicts that populations subject to relatively high extrinsic mortality should evolve relatively rapid rates of intrinsic decline (e.g. rapid actuarial senescence) (Medawar 1952, Williams 1957, Hamilton 1966, Kirkwood 1977; see Abrams 1993 and Caswell 2007 for important caveats). This widely-tested prediction has received mixed support based on among-species comparative studies

(Silvertown et al. 2001, Ricklefs 2010), within-species or -genera common garden experiments (Dudycha 2001, Reznick et al. 2004, Terzibasi Tozzini et al. 2013), and experimental evolution in laboratory environments (Stearns et al. 2000, Ackermann et al. 2007).

Given the mixed support for the most classic and widely-tested prediction from evolutionary theory on senescence (Williams et al. 2006), a great deal of variation in rates of senescence remains unexplained. This is particularly true when it comes to plants, which have historically been underrepresented in studies on the evolution of senescence (Monaghan et al. 2008, Salguero-Gómez et al. 2013). In contrast to most other taxonomic groups, the majority of plant species (at least angiosperms) seem not to exhibit actuarial senescence, though there is still a great deal of variation among plant species in age-trajectories of mortality (Silvertown et al. 2001, Baudisch et al. 2013), and plenty of plant species that do in fact senesce (e.g. Roach et al. 2009, Barks and Laird 2015).

Because rates of senescence often correlate with phylogeny (Ricklefs 2010, Baudisch et al. 2013), intraspecific comparisons are well suited for examining selective forces underlying variation in rates of senescence, but have thus far been mostly limited to animals (e.g. Reznick et al. 2004, Carlson et al. 2007, Terzibasi Tozzini et al. 2013). Apart from studies on the timing of senescence in annual plants (e.g. Griffith and Watson 2005), and among-population variability specifically in plant lifespans (Ehrlén and Lehtilä 2002, Hautekèete et al. 2002, van Dijk 2009), little is known regarding within-species variability in rates of senescence in plants.

Here I describe a common garden experiment examining variation in life expectancy and rates of senescence across 28 putative strains (derived from 23 populations) of the aquatic plant *Lemna minor*. My objective was to test whether *L. minor* displays genetically-based, among-strain variability in life history traits (with an emphasis on life expectancy and rate of senescence), and whether such variation correlates with variation in environmental characteristics at the sites of origin. I focused on environmental characteristics that are known to influence life history traits of *L. minor* on an ecological time scale (i.e. via phenotypic plasticity), including temperature (van der Heide et al. 2006), nutrient concentrations (Wangermann and Lacey 1955), and salinity (Haller et al. 1974).

Methods

OVERVIEW

In a laboratory environment, I tracked the complete life histories of over 1100 individual plants representing 28 'subsamples' (putative genetic strains) of *L. minor* originating from 23 sites in Alberta, Canada (for five of the 23 sites I studied two separate subsamples). Each subsample was clonally derived from a single plant and therefore represented a single genotype (whether each genotype was unique is a separate question that I will come back to).

I identified five specific research questions related to my objectives: (**Q1**) Is there among-subsample variation in life history traits in the common garden? The strength of the common garden approach is that it rules out environmental factors as a proximate explanation for trait variation (though the environment could still be

an 'ultimate' explanation for life history variation among subsamples). (**Q2**) As my experiment was split into two temporal blocks, I ask: within subsamples, are life history traits consistent between blocks? For example, do subsamples that have relatively high (low) values of a given life history trait in the first temporal block also have relatively high (low) values of that same trait in the second temporal block? Such consistency would be strong evidence for genetically-based, among strain variability in life history traits. (**Q3**) Are life history traits consistent among replicate subsamples from the same site? A lack of within-site consistency in life history traits would rule out local adaptation to environmental characteristics (at least at the site level) as a likely explanation for among-subsample variation. (**Q4**) Is there spatial autocorrelation in life history traits in the common garden (with respect to sites of origin), or environmental characteristics of the sites of origin? Spatial autocorrelation in life history traits could suggest local adaptation to environmental characteristics (if there is similar autocorrelation in the relevant environmental characteristic), or simply a spatial component to population genetic structure (i.e. populations in close proximity have relatively similar trait values because they share a relatively recent common ancestor). (**Q5**) Is there a direct relationship between life history traits in the common garden and environmental characteristics of the sites of origin?

STUDY SITES AND SAMPLING REGIME

My study sites initially included 25 wetlands in Alberta, Canada (Fig. 5-1) (two of the 25 sites were later dropped from my study, as explained below). The

sites were selected opportunistically based on the presence of *L. minor*, accessibility, and geographic distribution (I aimed for uniform geographic coverage of the southern two-thirds of Alberta). Most of the sites I sampled were small (<5 ha) and isolated (e.g. prairie potholes, sloughs), but I also sampled from marshes surrounding lakes and rivers, and open pools within bogs and fens.

In August 2013 I collected *L. minor* and water chemistry data from three subsample locations within each of the 25 sites, with each subsample location separated by at least 10 m. The 75 subsamples of *L. minor* were stored in coolers and taken back to the laboratory, where, from each subsample, I initiated an axenic, single-genotype stock culture as described in Appendix 3.

At each subsample location within each site, I measured specific conductance (a proxy for salinity) at the surface with a YSI probe (Model 30, YSI Inc., Ohio, USA), and collected surface water samples to assess total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP). Measurements and water samples were taken in areas where the water was at least 0.5 m deep, where possible. For each wetland, the three surface water subsamples were combined and filtered through a GF/F filter, then stored at 4°C. Quantification of TDN and TDP was performed by the Biogeochemical Analytical Service Laboratory at the University of Alberta.

COMMON GARDEN EXPERIMENT

I compared life history traits across the subsamples of *L. minor* in a common garden experiment, where the 'common garden' was a controlled, laboratory environment. Ideally, for each of the 25 initial sites, I would have studied plants

derived from each of the three subsamples, but there was a trade-off between the number of subsamples, and the number of plants per subsample that I could assess (due to limited time and space). I decided to have within-site replication for five randomly-selected sites (I studied two subsamples from each of these five 'replicated' sites), and used a single subsample for the remaining 20 'non-replicated' sites. I therefore initially studied 30 subsamples in total (recall that two of these 30 subsamples were later dropped from the experiment, as described below).

The common garden experiment was broken into two temporal blocks. In each block, I measured life history traits of 20 plants from each subsample. To limit within-subsample heterogeneity due to variation in parental age (e.g. Chapters 2 and 3), experimental plants in this study were all first offspring of first offspring of first offspring of an initial progenitor taken from the relevant stock culture. Starting with these initial progenitors, experimental plants were grown individually in 60 × 10 mm Petri dishes containing 10.5 mL of half-strength Schenk and Hildebrandt (S-H) growth medium (Sigma-Aldrich S6765), which I supplemented with sucrose (final concentration 6.7 g/L), yeast extract (0.067 g/L), and tryptone (0.34 g/L), to make microorganism contamination more easily detectable. Petri dishes were arranged on cookie-cooling racks and placed inside growth chambers at 24˚C with a 15:9 photoperiod and photosynthetic photon flux density at plant height of approximately 400 μmol m^{-2} s^{-1}. Assignment of Petri dishes to racks (and positions thereon), and racks to growth chambers (and positions therein) was done at random. To account for nutrient depletion and evaporation of the growth medium,

plants were aseptically transferred to new Petri dishes containing fresh growth medium every five days.

Each experimental plant was observed daily for the duration of its life. During each observation period, I noted how many daughters detached from each experimental plant since the previous day's observation period. Detached daughters were aseptically removed from the Petri dish and discarded.

SAMPLE LOSS

In the first temporal block of the common garden experiment, plants from two of the 25 sites grew in a 'clumpy' manner, which made it difficult for me to distinguish experimental plants from their descendants. Specifically, daughters would remain attached to their parent for a prolonged period of time, so that many generations might be growing together in a clump. Because I could not reliably track individual plants from these two sites, they were omitted from the experiment (thus limiting the common garden experiment to 23 sites, still five of which had within-site replication). From these 28 remaining subsamples comprising 1120 individual plants, 12 plants were discarded over the course of the experiment because I could not with certainty distinguish the focal plant from one of its descendants. Additionally, I was unable to measure total number of offspring for 29 of the remaining 1108 plants because, at some point in the focal plant's lifetime, I could not with certainty distinguish daughters from granddaughters or great-granddaughters.

ENVIRONMENTAL CHARACTERISTICS AND LIFE HISTORY TRAITS

The site-level environmental characteristics that I examined were surface water specific conductance (average of the three measurements for each site), TDN, TDP, and degree-days above 10˚C. Degree-days data were obtained from Environment Canada's Climate Normals database (years 1981-2010) based on the single climate station nearest to each site (see distances to nearest climate station in Fig. 5-2). The four environmental variables were all right-skewed, so I ln-transformed each variable prior to subsequent analyses. ln-Transformed TDN and TDP were strongly correlated, so I used principal component analysis to reduce them into a single principal component that explained 92% of their covariation. Environmental characteristics of the study sites are summarized in Table 5-1.

The life history traits I examined included lifespan, 'shape' of the mortality curve (*sensu.* Baudisch 2011), number of offspring, and frond surface area. I defined lifespan as the difference (in days) between the dates of birth and last reproduction, where date of birth was the day that a focal plant detached from its parent, and date of last reproduction was the day that a focal plant's final daughter detached. Shape of the mortality curve was measured as the proportion of individuals within a cohort surviving at least until the expected age at death (i.e. mean lifespan) for that cohort (Baudisch et al. 2013). Baudisch et al. show that, if morality rates are constant with age (i.e. no actuarial senescence), the proportion of individuals surviving to the expected age at death equals the inverse of the base of the natural logarithms (e^{-1}), or about 37%. If mortality rates decline with age (negative senescence), fewer than 37% of individuals will survive to the expected age at death due to the relatively high early mortality rate. In contrast, if mortality increases with age (actuarial

senescence), greater than 37% of individuals will survive to the expected age at death. My measure of shape is positively related to the rate of increase in mortality with age (i.e. the rate of actuarial senescence) within a cohort, and theoretically independent of the life expectancy for that cohort (Baudisch et al. 2013). The final life history trait – frond surface area – was measured in ImageJ v. 1.43u (Rasband 2012) using images captured with a microscope-mounted digital camera. These images were captured late in a focal plant's life when it had no attached daughters.

DATA ANALYSIS

All analyses were carried out in R v. 3.1.2 (R Core Team 2015). Among my various statistical analyses, there was variation in the level at which I modeled life history response variables: either at the level of the individual plant, subsample by temporal block, subsample (blocks pooled), or site (blocks and replicate subsamples pooled). Three of the four life history traits (lifespan, total offspring, and frond surface area) were fundamentally plant-level variables, so their transformation to higher levels was based on median values. For example, the site-level lifespan for site *i* was simply the median lifespan of the 40 plants from that site (or 80 plants if site *i* was one of the five replicated sites). The shape parameter was somewhat different from the other traits in that shape is a property of a cohort, not an individual plant. Thus, shape was always modeled at levels higher than the individual plant, and simply based on the cohort of individuals implied by the level of focus (e.g. the 20 plants in subsample *j,* block *k*). For some analyses, I used

bootstrapping to estimate the sampling distribution of the shape parameter, either within a subsample (Q1), or subsample by block (Q3).

My analyses follow directly from the five questions listed in the *Overview* section. First, to understand whether life history traits varied among the 28 subsamples of *L. minor* (Q1), I fit random-effect models describing plant-level (or for shape, bootstrapped) life history traits as a function of a subsample-specific random intercept, using the *lme* function in the R package NLME. I then used the *varcomp* function in the package APE to estimate the among-subsample proportion of the total variance (also called intraclass correlation, ICC) in each life history trait. I used a similar approach to estimate the consistency in life history traits among replicate subsamples from the same site (Q3), except that, for this analysis, I modeled subsample nested within site (here I was interested in comparing the among-*subsample* proportion of variance to the among-*site* proportion of variance). This latter analysis was limited to a reduced dataset comprising only the five sites that were replicated in the common garden experiment. For the above-described analyses, I used bootstrapping to generate 5000 shape values for each cohort (Q1: cohort = subsample; Q3: cohort = subsample by block), which served as the response variable in my random-effect models pertaining to the shape parameter. This application of the bootstrap might seem unsuitable from a hypothesis-testing standpoint (where results may depend on bootstrap sample size), but here I am only using the statistical model as a tool to estimate variance components, which are unrelated to bootstrap sample size (Fig. 5-3).

To assess whether life history traits were consistent across the two temporal blocks (Q2), I examined the correlation (focusing on R^2 values) between life history traits for each subsample in block 1 versus block 2. Life history traits in this analysis were modeled at the level of subsample by block.

I used Moran's I to measure the degree of spatial autocorrelation (with respect to sites of origin) in site-level life history traits and environmental characteristics (Q4). I calculated Moran's I and relevant statics using the *Moran.I* function in the R package APE, based on an among-site great-circle distance matrix created using the *distCosine* function in the GEOSPHERE package.

Finally, to assess whether life history traits in the common garden were related to environmental characteristics of the sites of origin (Q5), I fit multilevel mixed-effect models relating plant-level life history traits (lifespan, total offspring, and frond surface area) to the three site-level environmental characteristics (degree-days, nutrient PC1, and conductivity; modeled as fixed effects with no interactions). These models included a site-specific random intercept term, and were again fit using the *lme* function in the package NLME. I used likelihood ratio tests to examine the evidence for relationships between life history traits and environmental characteristics. For the shape parameter, I used ANOVA to assess the relationship between site-level shape values and the three site-level environmental characteristics (again modeled as fixed effects with no interactions).

Results

There was little variation in survivorship trajectories among the 28 subsamples of *L. minor* (Fig. 5-4). Correspondingly, the among-subsample proportions of total variance for lifespan and shape were low – 10% and 30%, respectively (Fig. 5-5a,b). The among-subsample proportions of total variance for total offspring and frond surface area were greater, at 45% and 71% respectively (Fig. 5-5c,d).

I observed relatively high within-subsample consistency in life history traits across the two temporal blocks, except with respect to the shape parameter, which was not at all consistent between blocks (Fig. 5-6).

Considering only the five sites that were replicated, there was very little consistency in life history traits among replicate subsamples from the same site (Fig. 5-5; notice that replicates do not cluster together). Specifically, in models where subsample was nested within site, the among-*site* proportion of total variance in life history traits was less than 1% for lifespan, total offspring, and frond size, and 9% for the shape parameter. Among-*subsample* variation for the five replicated sites was greater, and similar to that for the full analysis (i.e. Q1), with values of 7% (lifespan), 32% (shape), 52% (total offspring), and 79% (frond surface area).

There was no significant spatial autocorrelation in any of the site-level life history traits that I examined (Table 5-2). The only environmental characteristic showing spatial autocorrelation was degree-days above 10°C (Table 5-2).

Life history traits in the common garden were not related to environmental characteristics of the sites of origin, except for a negative relationship between frond surface area and nutrient concentrations (Fig. 5-7, Table 5-3).

Discussion

My objective was to test whether *L. minor* displays genetically-based, among-strain variation in life history traits (with an emphasis on life expectancy and rate of senescence), and whether such variation correlates with variation in environmental characteristics at the sites of origin. I found little evidence for among-strain variation either in life expectancy or rate of senescence (i.e. the shape parameter), given that neither of these traits exhibited both substantial variation among subsamples, and within-subsample consistency across temporal blocks. However, the other two life history traits (total offspring and frond surface area) did exhibit both variation among, and consistency within subsamples – indicative of genetically-based, among-strain variability.

There were no relationships between life history traits in the common garden and environmental characteristics of the sites of origin apart from a relatively weak negative relationship between frond surface area and nutrient concentrations (ln-TDN and ln-TDP combined into a single principal component). Interestingly, under laboratory conditions, frond size actually *increases* with increasing nitrogen concentration (Wangermann and Lacey 1955), so the genetic relationship I observed between frond size and nutrient concentration was opposite to their relationship based on phenotypic plasticity. In any case, I did not observe consistency in frond surface area (or any other life history trait) among replicate subsamples from the same site, which suggests that the relationship between surface area and nutrient concentrations may have been spurious. Alternatively, the lack of within-site

consistency in surface area may have been a function of sampling error, given that only five sites were replicated. The five replicated sites (which were selected randomly from the initial 25 sites) all happened to have above-average nutrient concentrations (Table 5-1). Even if there were in fact a relationship between nutrient concentrations and frond size at the (statistical) population level, the chance of observing within-site consistency based on a small sample of sites over a narrow range of nutrient concentrations may have been low.

WERE SUBSAMPLES GENETICALLY UNIQUE?

As my study was concerned with genetically-based variation in life history traits (and environmental correlates thereof), a premise of my study design was that 'subsamples' generally represented unique genotypes (though it would not be inferentially problematic if some subsamples were not unique). Previous population genetic studies based on allozyme variation documented relatively high levels of genetic variability in *L. minor*, both within and among populations. For example, Vasseur et al. (1993) observed 157 unique genotypes of *L. minor* among eight small ponds in Ontario, Canada (maximum inter-pond distance was 12 km). In that study, the average number of unique genotypes per pond was 19.6, and the average number of ponds per genotype was 1.8 (indicating relatively high among-population differentiation). Cole and Voskuil (1996) found an average of 4.0 genotypes per population based on a study of 11 populations of *L. minor* in Minnesota (maximum inter-population distance was 281 km), and also documented relatively high levels of among-population differentiation (mean F_{ST} = 0.4). Given that my study covered a

much larger spatial scale than the studies described above (mean distance from a given site to its nearest neighbour was 75 km, and maximum inter-site distance was 856 km), I had the *a priori* expectation that most of the subsamples would be genetically unique.

Irrespective of *a priori* expectations, my results suggest that subsamples were in fact (at least mostly) genetically unique, given the high within-subsample consistency in life history traits (except for the shape parameter) across temporal blocks (Fig. 5-6). I can rule out environmental explanations for this consistency given that my study was a fully-randomized common garden experiment. Epigenetic explanations are also unlikely given that epigenetic transmission is usually limited to just a few generations (Hauser et al. 2011). My experiment began about 10 months (~43 generations assuming a mean generation time of 7 days; calculated based on data in Chapter 2) after stock cultures for each subsample were initiated, and the two temporal blocks were initiated about two months (~9 generations) apart. Furthermore, the lack of within-site consistency in life history traits (despite consistency within subsamples across blocks), suggests that even replicate subsamples from the same site were (at least mostly) genetically unique.

CONCLUSIONS

Empirical research on the evolution of senescence has largely focused on one theoretical prediction – that populations subject to high extrinsic mortality will evolve rapid rates of intrinsic decline (reviewed in Williams et al. 2006). Support for this prediction is mixed, and as others have pointed out (Abrams 1993, Caswell

2007), the prediction does not actually follow from formal theory anyway. Thus, notwithstanding the many known life history correlates of life expectancy (e.g. body mass, Promislow 1991; age at maturity, Purchase et al. 2005; initial growth rate, Ricklefs and Scheuerlein 2001; reproductive effort, reviewed in Roff 1992, pp. 157-163), much remains to be learned about the extrinsic forces underlying variation in rates of senescence. This is especially true when it comes to plants, which have historically been underrepresented in research on the evolution of senescence. In contrast to most other taxonomic groups, a majority of plants do not seem to exhibit actuarial senescence, though there is still a great deal of variation among plant species in age-trajectories of mortality (Silvertown et al. 2001, Baudisch et al. 2013). The current study is to my knowledge the first to examine variation *within* a plant species in rates of senescence (though others have demonstrated within-species variation in plant lifespans; e.g. Ehrlén and Lehtilä 2002, Hautekèete et al. 2002, van Dijk 2009). At least within my study species (*Lemna minor*), life expectancy and rates of senescence appear to be highly conserved over a wide geographic range, despite variability in other life history traits including plant size and number of offspring. *L. minor* is perhaps then a poor candidate for further study on intraspecific variation in rates of senescence. Nonetheless, given the incredible diversity in age-trajectories of mortality among plant species, our understanding of the evolutionary forces underlying variation in rates of senescence will benefit from an increased focus on plants.

Table 5-1. Physical and environmental characteristics of the study sites. Study sites are arranged from lowest to highest nutrient concentration, based on the first principle component of TDN and TDP (column 'Nut PC1'). The five replicated sites (shaded grey) all happened to have above-average nutrient concentrations.

Site	Lat	Lon	Cond	TDN	TDP	Nut PC1	Deg Days	Climate Stn	Stn Dist
wht	54.06	-115.83	619	737	8	-3.4202	563.0	Whitecourt A	8.3
elk	49.66	-110.27	404	459	20	-2.7053	1079.1	Medicine Hat A	50.6
prk	49.81	-112.92	332	621	44	-1.8615	767.6	Monarch	16.6
cld	49.73	-112.62	468	1236	49	-1.5379	874.0	Lethbridge A	17.2
eds	53.61	-115.95	393	1752	59	-1.2497	483.2	Shining Bank	27.3
hhl	53.74	-112.07	590	2216	63	-1.1119	642.9	Vegreville	27.1
cas	54.66	-112.51	219	2872	58	-1.1067	630.6	Athabasca 2	68.1
yng	55.13	-117.57	222	438	119	-1.0320	617.4	Valleyview RS	20.5
hwy	54.01	-113.15	940	1716	105	-0.7107	548.6	Redwater	2.6
slv	55.41	-114.80	424	1844	109	-0.6521	633.6	Wabasca RS	86.7
wan	55.20	-112.54	194	1216	130	-0.6194	539.8	Calling Lake RS	41.0
win	55.61	-116.76	1339	2724	110	-0.5178	649.1	Ballater	27.9
skf	49.37	-111.80	743	661	235	-0.2553	966.7	Foremost	28.7
anz	56.45	-111.04	140	2000	169	-0.2108	654.8	Fort McMurray A	24.9
bar	54.15	-114.46	636	3648	159	-0.0749	580.4	Campsie	13.9
stn	55.20	-119.06	187	1904	255	0.1628	599.8	Grande Prairie A	11.7
dbn*	49.03	-112.75	1328	2880	399	0.7199	744.8	Cardston	36.3
keh	54.12	-110.82	731	2220	529	0.9031	573.3	St Lina	45.5
val	55.17	-117.16	190	1920	586	0.9532	617.4	Valleyview RS	13.6
tay	49.03	-113.12	1195	2848	696	1.2431	744.8	Cardston	12.7
mch	49.54	-112.56	3043	4240	1160	1.8549	874.0	Lethbridge A	18.8
dwd	52.86	-110.76	334	3716	1720	2.1853	685.3	Fabyan	20.4
han*	51.50	-112.06	618	5960	2680	2.7573	721.3	Craigmyle	33.4
pat	50.69	-111.67	2812	4040	4280	3.0752	941.7	Jenner	34.1
cam	52.89	-112.71	271	3884	5010	3.2117	663.6	Camrose	17.0

Site gives the abbreviated name of each study site (asterisks identify the two sites that were dropped from the study); **Lat** and **Lon** are given in decimal degrees; **Cond** is specific conductance (μS cm^{-1} at 25°C); **TDN** is total dissolved nitrogen and **TDP** is total dissolved phosphorus (both in μg L^{-1}); **Nut PC1** is the first principle component of ln-transformed TDN and TDP; **Deg Days** is degree days above 10°C; **Climate Stn** is the name of the Environment Canada climate station nearest to each study site; **Stn Dist** is the great-circle distance (in km) between the climate station and study site.

Table 5-2. Moran's I tests for spatial autocorrelation in site-level life history traits in the common garden, and environmental characteristics of the sites of origin.

	z(Moran's I)*	P-value
Life history trait		
Lifespan	-0.7	0.50
Shape parameter	-0.4	0.68
Total offspring	0.8	0.43
Frond surface area	0.2	0.86
Environmental characteristic		
ln-Degree-days	7.3	3.5×10^{-13}
PC1(ln-TDN, ln-TDP)	-0.1	0.91
ln-Conductivity	1.5	0.14

* The Moran's I test statistic is uninformative without comparison to the expected value, so I present z-transformed Moran's I: (observed – expected) / SD. Positive values indicate positive autocorrelation, and negative values indicate negative autocorrelation (dispersion).

Table 5-3. Statistical results from models comparing life history traits in the common garden to environmental characteristics of the sites of origin.

Life history trait (response variable)	Environmental Characteristic	Test Statistic	P-value
Lifespan*	ln-Degree-days	$D = 0.01$	0.92
	PC1(ln-TDN, ln-TDP)	$D = 2.58$	0.11
	ln-Conductivity	$D = 0.08$	0.77
Shape parameter†	ln-Degree-days	$F = 0.66$	0.45
	PC1(ln-TDN, ln-TDP)	$F = 0.83$	0.37
	ln-Conductivity	$F = 0.29$	0.60
Total offspring*	ln-Degree-days	$D = 0.70$	0.40
	PC1(ln-TDN, ln-TDP)	$D < 0.01$	0.98
	ln-Conductivity	$D = 0.29$	0.59
Frond surface area*	ln-Degree-days	$D = 0.40$	0.55
	PC1(ln-TDN, ln-TDP)	$D = 7.48$	0.006
	ln-Conductivity	$D < 0.01$	0.96

* Multilevel mixed-effect models relating plant-level life history traits to site-level environmental characteristics (modeled as fixed effects with no interactions). Testing of fixed effects was based on likelihood ratio tests (test statistic is the likelihood ratio, D).
† Modeled with ANOVA. Response variable modeled at the site-level.

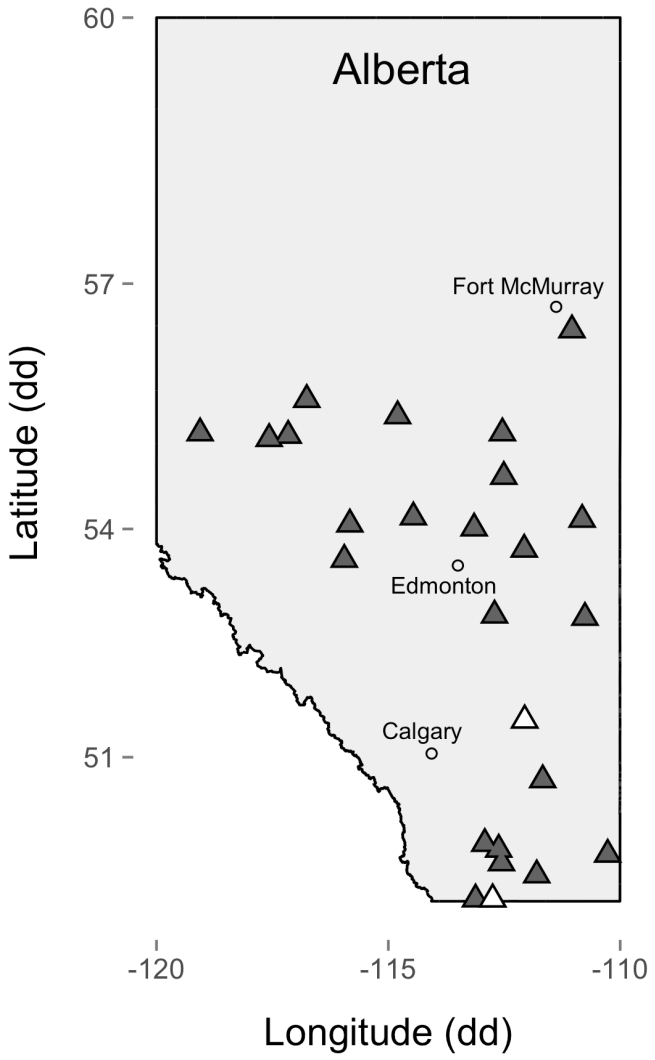

Figure 5-1. Sites of origin for the 25 populations of *L. minor* initially sampled for my common garden experiment. The two sites that were dropped from the experiment are depicted as white triangles, and the remaining 23 sites as grey triangles.

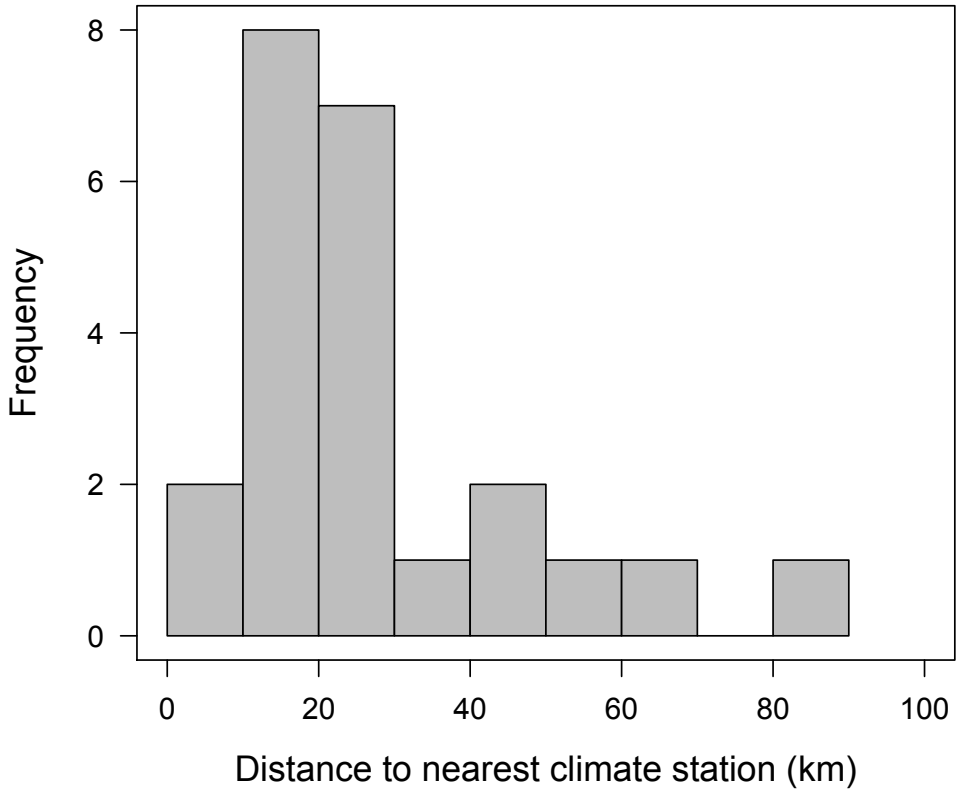

Figure 5-2. Histogram of distances from study sites to the nearest climate station.

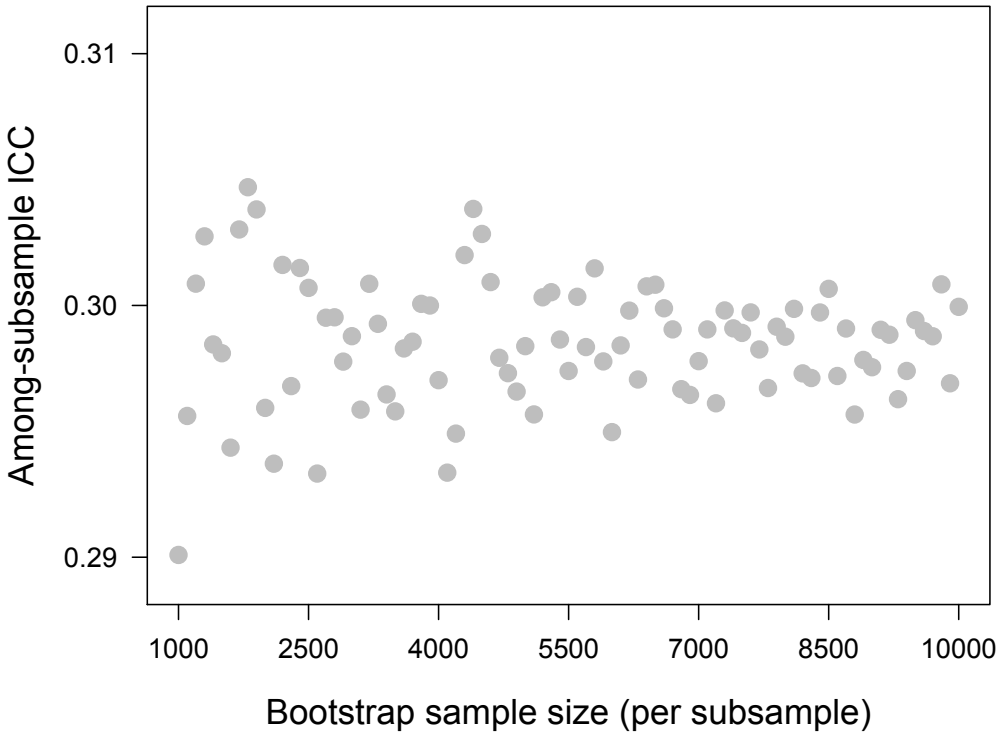

Figure 5-3. Among-subsample intraclass correlation coefficients (ICC) for a range of bootstrap sample sizes (i.e. number of shape values bootstrapped for each subsample). Here, ICCs estimate the among-subsample proportion of total variance in the shape parameter. The figure demonstrates that ICCs were estimated with a high degree of precision at my selected bootstrap sample size of $N_{boot} = 5000$ per subsample, and that ICCs are not dependent on bootstrap sample size.

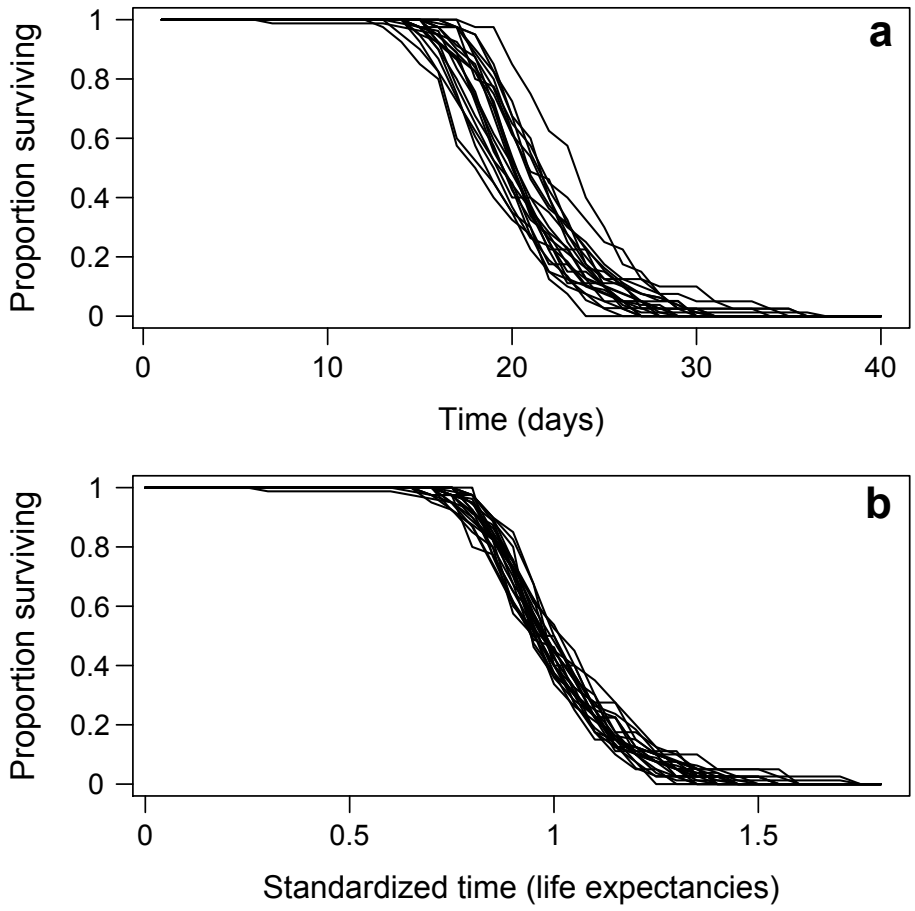

Figure 5-4. Survivorship trajectories in the common garden for 28 subsamples of *L. minor*. Trajectories in the top panel are based on regular units of time (days), whereas trajectories in the bottom panel are based on standardized units of time (days divided by the life expectancy for each subsample). Comparing standardized survivorship trajectories allows for a better appreciation for how the shape of survival trajectories varies (or in this case, does not vary) among groups.

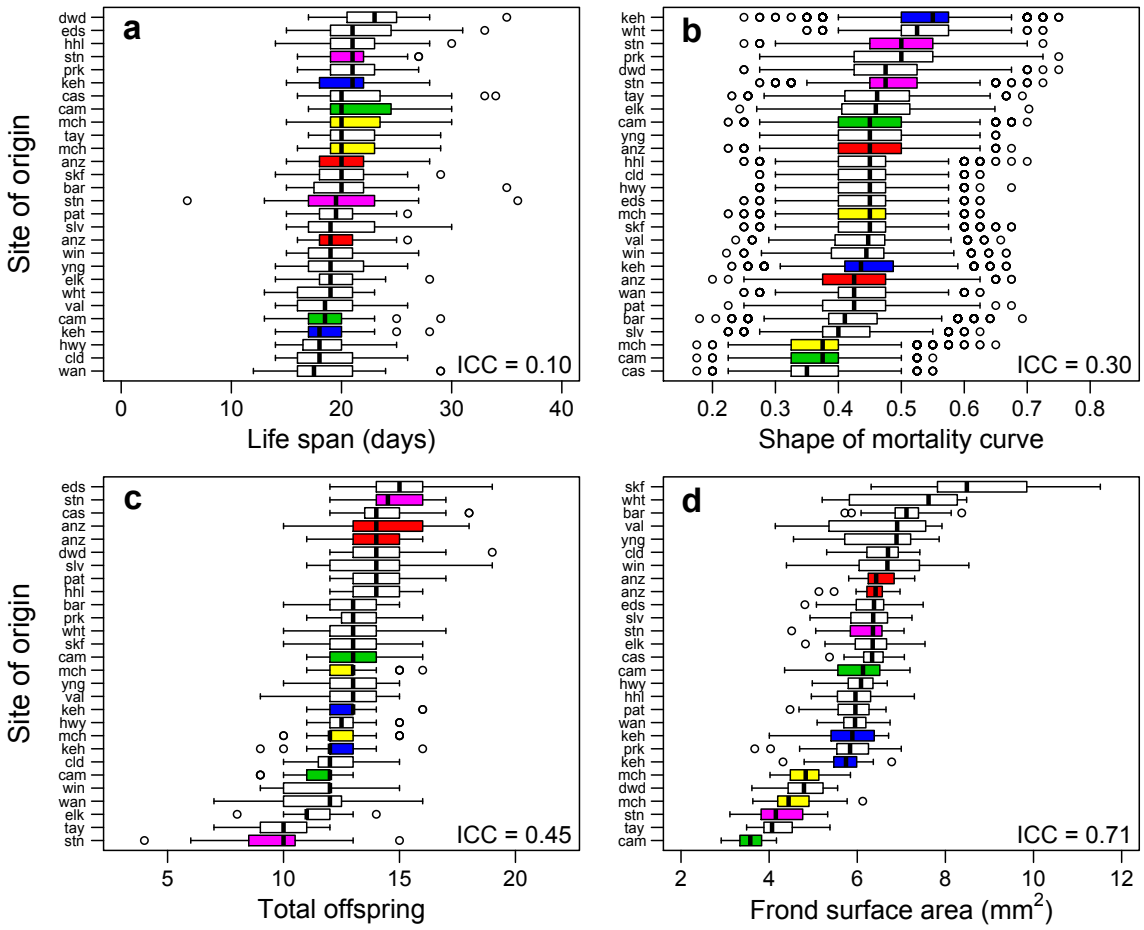

Figure 5-5. Life history traits in the common garden by site of origin. Traits include lifespan (**a**), shape of the mortality curve (**b**), total number of offspring (**c**), and frond surface area (**d**). Within-site replicates are depicted with matching colours, and non-replicated sites are in white. Higher shape values in panel B indicate a more rapid acceleration in mortality, independent of life expectancy. Intraclass correlation coefficients (ICCs) represent the among-subsample proportion of total variance in a given life history trait. Boxes depict the median and first and third quartiles, and whiskers extend to the lowest and highest data points within 1.5 times the interquartile-range of the first and third quartile, respectively.

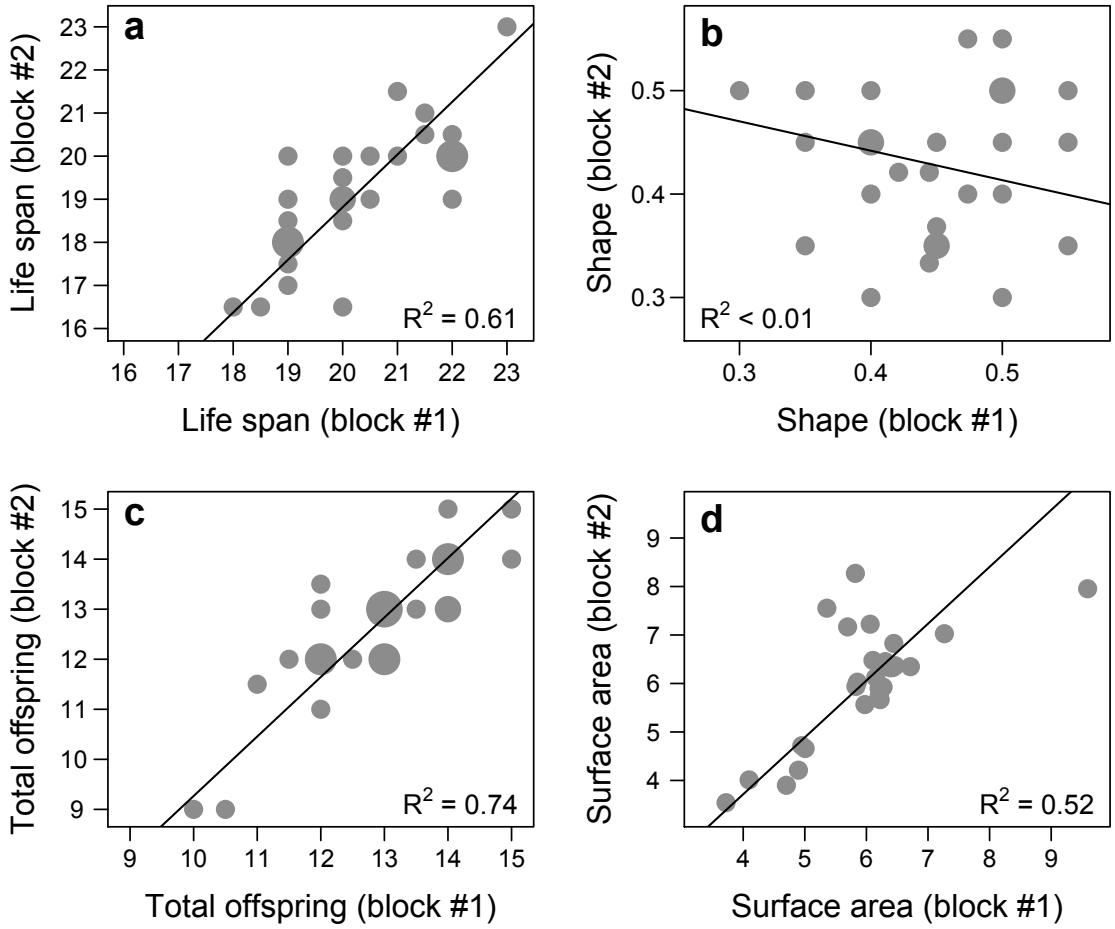

Figure 5-6. Within-subsample consistency in life history traits between two temporal blocks. Traits include lifespan (**a**), shape of the mortality curve (**b**), total number of offspring (**c**), and frond surface area (**d**). Point area is proportional to the number of observations at a given set of coordinates. Best-fit lines are based on total least squares regression.

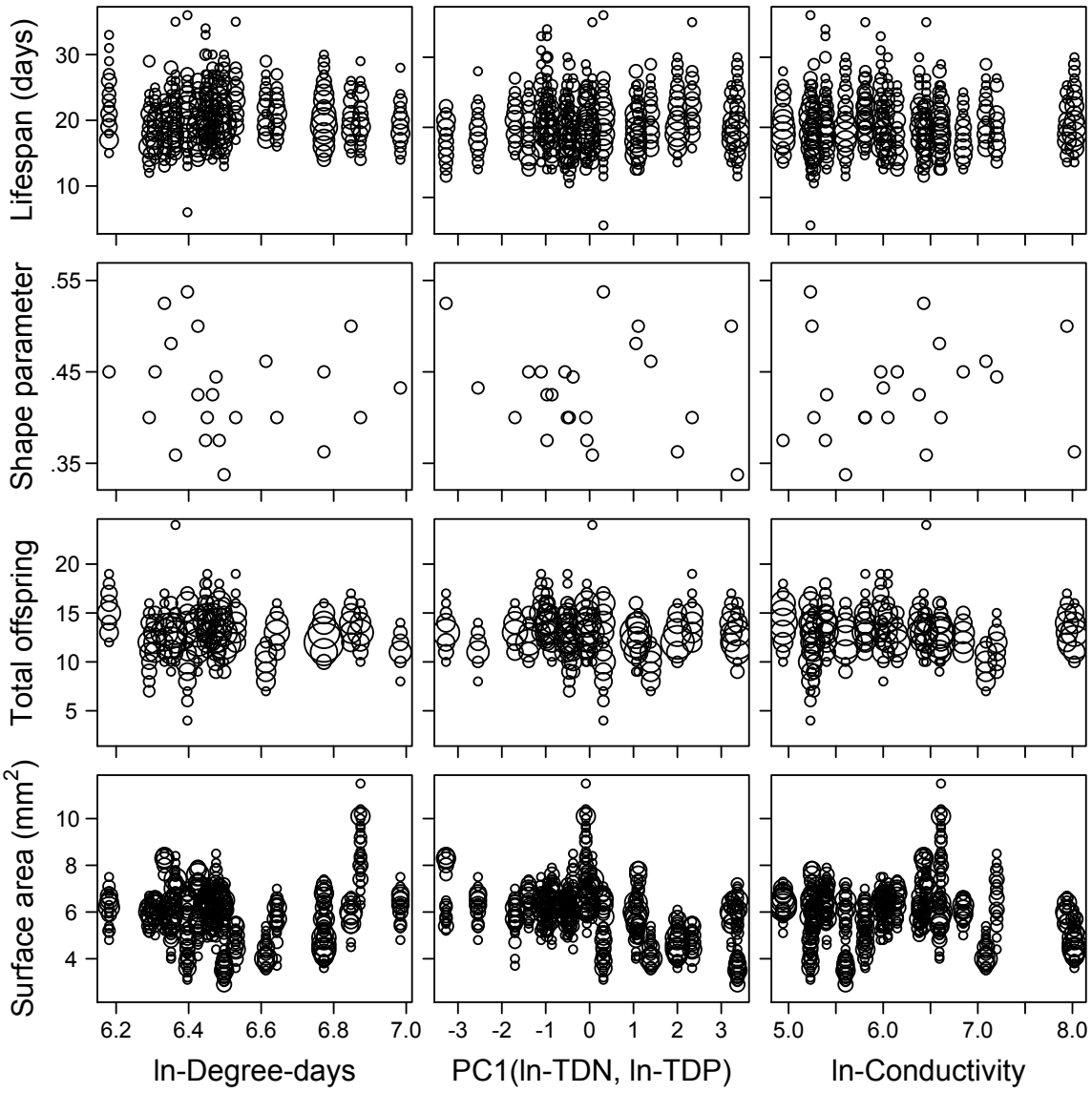

Figure 5-7. Life history traits in the common garden versus environmental characteristics at the sites of origin. Panels depict life history traits determined at the level of individual plants, except for the shape parameter, which is depicted here as a site-level trait (see Methods for further details). Point area is proportional to the number of observations at a given set of coordinates.

CHAPTER 6. GENERAL DISCUSSION

PARENTAL AGE EFFECTS AND THE FORCE OF NATURAL SELECTION

It has long been recognized that an individual's phenotype may depend on the phenotype of its parent(s) above and beyond the expected relationship due to direct genetic contributions (commonly referred to as a 'maternal effect'; reviewed in Roach and Wulff 1987, Kirkpatrick and Lande 1989, Bernardo 1996, Mousseau and Fox 1998). There has been a great deal of recent interest in the ecological and evolutionary consequences of such effects (e.g. Beckermann et al. 2002, Räsänen and Kruuk 2007, Marshall et al. 2010). Parental age effects – a subset of maternal effects (or more generally 'parental effects') – have a similarly long history (e.g. Bell 1918, Lansing 1947, 1948, Parsons 1964), and have received recent attention with respect to evolutionary theory on senescence (Kern et al. 2001, Priest et al. 2002, Pavard et al. 2007a,b, Pavard and Branger 2012, Gillespie et al. 2013a). Most classic theory on the evolution of senescence makes the simplifying assumption that all offspring are of equal quality, but this assumption is clearly violated if offspring quality is a function of parental age. As others have pointed out (e.g. Kern et al. 2001, Priest et al. 2002), age-related declines in offspring quality are conceptually similar to age-related declines in survival and fecundity (i.e. demographic senescence), and may have similar evolutionary consequences (Pavard et al. 2007a,b, Pavard and Branger 2012, Gillespie et al. 2013a).

In Chapters 2-4, I characterized parental age effects in *L. minor*, and developed a population projection model to investigate the evolutionary

consequences of such effects (i.e. investigate how parental age effects might modify the force of selection acting on age-specific vital rates). First, in Chapter 2, I characterized age-trajectories of the three major demographic components of fitness in *L. minor*: survival, fecundity, and offspring quality. All three components declined strongly with increasing age. This result extends and clarifies earlier research on senescence in *L. minor*, which (i) documented parental-age-related declines in offspring size, lifespan, and total reproductive output (Wangermann and Ashby 1950, Wangermann 1952, Ashby and Wangermann 1954), and (ii) provided some evidence for age-related declines in survival and fecundity (Ashby et al. 1949, Wangermann and Lacey 1955). Next, in Chapter 3, I examined whether parental age effects in *L. minor* carry over across multiple generations. I found that parental age effects on offspring size did carry across generations, but effects on offspring fitness did not. This result had important implications for Chapter 4, where I developed a population projection model incorporating (non-multigenerational) parental age effects on offspring vital rates. In agreement with recent theory (Pavard et al. 2007a,b, Pavard and Branger 2012, Gillespie et al. 2013a), my analyses in Chapter 4 suggest that the parental age effect in *L. minor* should act to increase the force of selection on early age classes and reduce the force of selection on late age classes, compared to what is expected in the absence of parental age effects (i.e. parental age effects should lead to a steeper decline in the force of selection with age). Classic evolutionary theory suggests that such an effect will pave the way for the evolution of a more rapid rate of senescence.

PROXIMATE EXPLANATIONS FOR PARENTAL AGE EFFECTS

Though my thesis is not specifically concerned with proximate explanations for parental age effects, some of my findings potentially relate to proximate causation anyway, and therefore warrant some discussion.

In general, proximate explanations for parental age effects can be grouped into three broad hypotheses: (**1**) mutation accumulation in parental reproductive tissues (Crow 1997), (**2**) the accumulation and somatic transfer of deleterious compounds from parents to offspring (Ashby and Wangermann 1951), or (**3**) declines in the quality of the environment in which offspring develop (e.g. declines in parental care or provisioning; Fox 1993). Mutation accumulation seems a particularly unlikely explanation here given that reproduction in *L. minor* is primarily asexual, which potentially renders it subject to Muller's ratchet and mutation meltdown (Lynch et al. 1993). Furthermore, if parental age effects in *L. minor* were due to mutation accumulation, I would expect such effects to accumulate over multiple generations, but this was not the case (at least with respect to offspring fitness; Chapter 3). I did observe a multigenerational effect of parental age on offspring size (Chapter 3), but previous work suggests that lineages can recover from such effects (Wangermann and Ashby 1951). This recovery is inconsistent with mutation accumulation as an explanation for parental age effects.

The remaining proximate hypotheses for parental age effects (2 and 3 above) concern the accumulation of deleterious compounds and changes in the developmental environment, respectively. Here, I propose a potential explanation for parental age effects specific to *L. minor* that falls within the scope of hypothesis 3.

In particular, Lemon and Posluszny (2000) found that when a daughter frond detaches from its parent, a small amount of connective tissue (deriving from a structure called the stipe) is left behind in the parent's meristematic pocket. They report, "after several daughter fronds have been produced, a large amount of stipe tissue will have accumulated in the pockets" (p. 743). Thus, I hypothesize that the accumulation of stipe tissue in the meristematic pockets of *L. minor* fronds progressively constricts or otherwise modifies the growth environment experienced by successive daughters, which may play a role in the age-related declines in offspring size or fitness observed in Chapters 2 and 3.

The stipe-accumulation hypothesis, however, does not obviously entail multigenerational effects, potentially making it inconsistent with results from Chapter 3 (at least with respect to frond size; effects of parental age on offspring fitness were not multigenerational). That said, I can easily imagine auxiliary hypotheses that would lead to a multigenerational effect: for example, if, independent of stipe accumulation, there exists a mechanism leading to a correlation between parent and offspring size (i.e. late-produced offspring will be small because they developed in a constricted environment due to stipe-accumulation, and *their* offspring will be small simply because the parent was small). Studies that examine parental-age-related variation in both demographic and physiological traits will likely be needed to test the above-described hypotheses.

A second result from my thesis that potentially bears on the proximate cause of parental age effects in *L. minor* is the finding that frond size and fitness both initially *increased* with parental age prior to their later declines. Specifically, in

Chapter 3, frond size and fitness both peaked at an immediate birth order of $N_P = 3$, and frond size similarly peaked at an ancestral birth order of N_P-N_P-N_P = 3-3-3 (recall that offspring fitness was unaffected by ancestral birth order in Chapter 3). In Chapter 2, the fitness of right-produced offspring peaked at birth order $N_R = 2$ and declined thereafter; however, in Chapter 2, the fitness of left-produced offspring, and the size of both right- and left-produced offspring declined monotonically with parental age (Fig. 6-1). Results from previous studies are similarly conflicting. For example, Claus (1972) found that frond size in *L. perpusilla* peaked at a parental age of about 5 days and then progressively declined, whereas Ashby and colleagues generally observed monotonic declines in offspring size with increasing parental age (Ashby et al. 1949, Wangermann and Ashby 1951, Wangermann 1952, Ashby and Wangermann 1954). These conflicting results suggest that whether there is an initial increase in frond size or fitness with increasing birth order is perhaps strain- or environment-dependent.

What could be the proximate cause of an initial increase in offspring quality with increasing birth order? Hypotheses 1 and 2 for parental age effects, and the stipe-accumulation hypothesis (all described above) are unlikely candidates because mutations, somatic damage, and stipe tissue would only ever accumulate over time (at least on average), so the resultant decline in offspring quality should be monotonic under these hypotheses. I therefore suggest that the initial increase in offspring quality with birth order likely relates to hypothesis 3 (excluding stipe accumulation) – some unique aspect of the environment in which first-offspring develop. I note here that, in addition to the initial increase in offspring quality

observed here and in and other studies, I have consistently observed – in many strains of *L. minor* – a morphological difference between first offspring (N_P = 1) and all subsequent offspring. Specifically, in my experience, first offspring are never bilaterally symmetrical (their distal end is angled), whereas all subsequent offspring are symmetrical (their distal end is rounded) (Fig. 6-2). Whether this observation relates to the parental age effects on offspring size or fitness is unclear, but it does point to first and subsequent offspring experiencing somewhat different developmental environments, corresponding to hypothesis 3 above.

AMONG-POPULATION VARIATION IN PATTERNS OF SENESCENCE

There is extensive variation in rates and patterns of senescence in nature, both within and among species (within-species: Reznick et al. 2004, van Dijk 2009, Terzibasi Tozzini et al. 2013; among-species: Silvertown et al. 2001, Ricklefs 2010, Baudisch et al. 2013, Jones et al. 2014). The theoretical tool most commonly invoked to explain this variation is Williams' (1957) prediction that relatively high extrinsic mortality should favour the evolution of relatively rapid rates of intrinsic decline (i.e. rapid demographic senescence). Support for this prediction has been mixed (reviewed in Williams et al. 2006), and perhaps more importantly, the prediction does not actually follow from formal theory anyway (Abrams 1993, Caswell 2007). Thus, relatively little is known about the extrinsic factors underlying variation in rates of senescence.

In Chapter 5, I described a common garden experiment examining among-population variation in rates of actuarial senescence in *L. minor*, and environmental

correlates thereof. I found little among-population variation in life expectancy or rate of actuarial senescence, despite variation in other life history traits including plant size and total reproductive output. As my study was not testing any formal theoretical predictions regarding extrinsic forces underlying variation in rates of senescence, the main conclusion is simply that *L. minor* is perhaps a poor candidate for further study on intraspecific variation in patterns of senescence. That said, since life expectancy is known to vary extensively among populations of other plant species (e.g. Ehrlén and Lehtilä 2002, Hautekèete et al. 2002, van Dijk 2009), the question of why life expectancy is seemingly highly conserved in *L. minor* naturally arises. Understanding why life expectancy is variable within some species while conserved within others may provide important insight into the extrinsic forces that shape patterns of senescence.

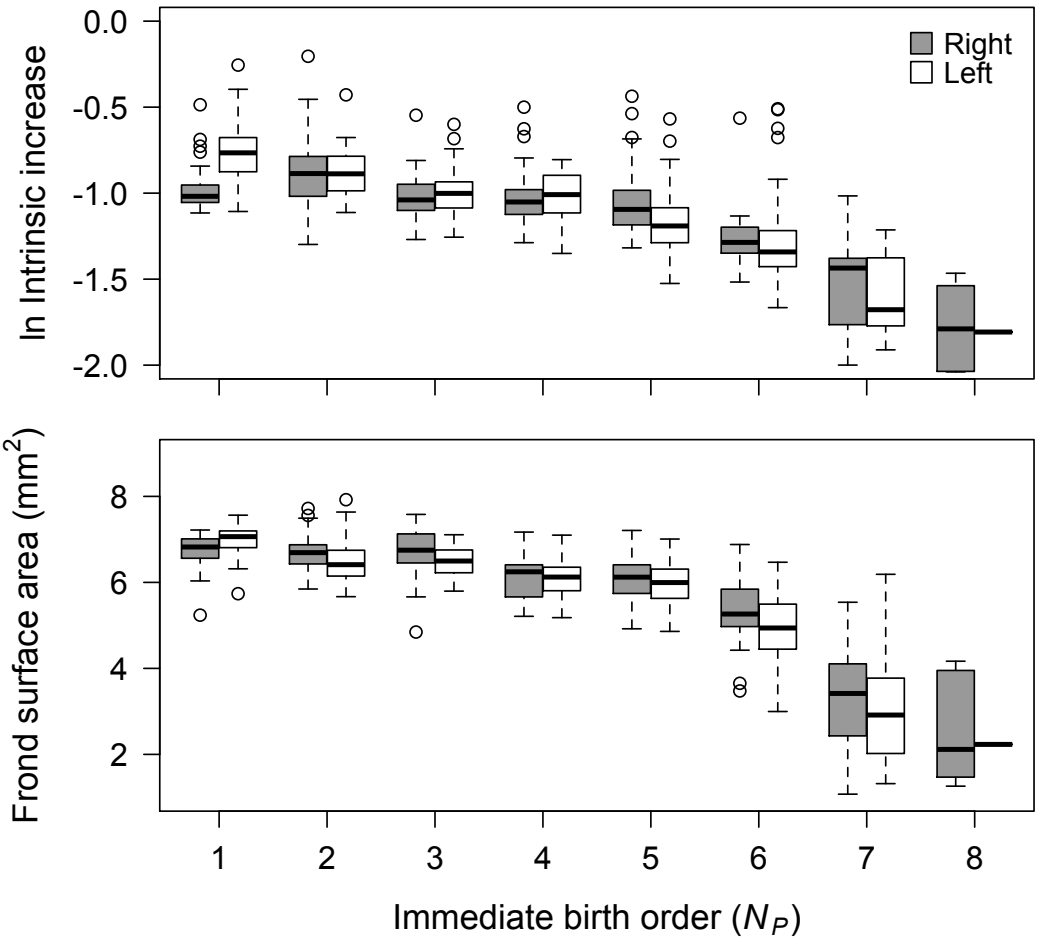

Figure 6-1. ln-Transformed individual intrinsic rates of increase (top panel) and frond surface area (bottom panel) versus pocket-specific (i.e. from the right vs. left meristematic pocket) immediate birth order. Data are from Chapter 2, and represent all 542 of the offspring detached from 41 parental fronds. Boxes depict the median and first and third quartiles, and whiskers extend to the lowest and highest data points within 1.5 times the interquartile-range of the first and third quartile, respectively.

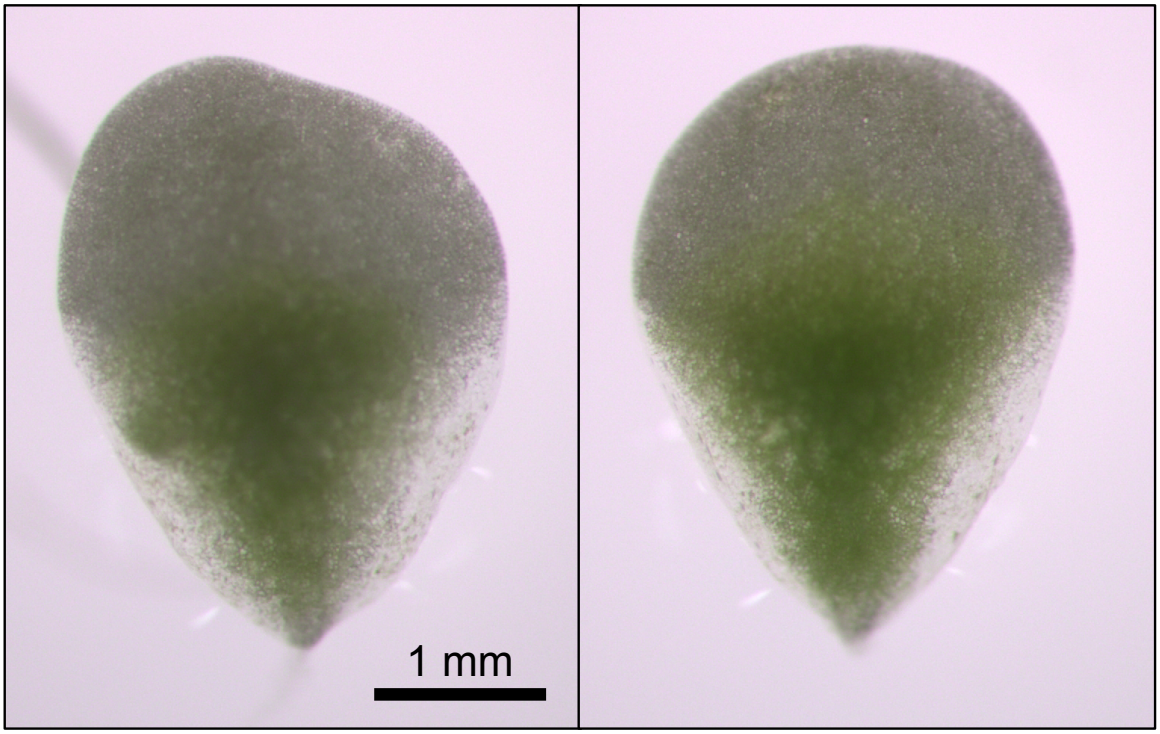

Figure 6-2. Comparison of the morphology of representative first (N_P = 1; left panel) and subsequent (N_P > 1; right panel) daughter fronds of *L. minor*. In my experience, first daughters are almost always 'angled' at the distal end and are therefore not bilaterally symmetrical, whereas all subsequent daughters have a rounded distal end and therefore appear symmetrical.

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APPENDICES

APPENDIX 1: PUBLICATION INFORMATION

A modified version of Chapter 2 was previously published in the journal Functional Ecology. It is reprinted here with permission from Jon Wiley and Sons (License Number: 3617251294915).

Citation:
Barks, P.M., and Laird, R.A. 2015. Senescence in duckweed: age-related declines in survival, reproduction and offspring quality. Functional Ecology 29:540-548.

URL:
http://onlinelibrary.wiley.com/doi/10.1111/1365-2435.12359/full

APPENDIX 2: METHOD FOR CREATING AN AXENIC STOCK CULTURE (CHAPTER 3)

My protocol for creating a sterile stock culture was based on Hillman (1961, p. 236-237). First, I 'pre-cultured' wild-collected fronds for 24 hours in Modified Hoagland E+ liquid growth medium supplemented with sucrose, yeast extract, and tryptone (recipe in Environment Canada 2007, p. 12-14). This pre-culture period was meant to encourage microorganism growth and spore germination. After the 24-hour pre-culture, I submerged fronds individually in varying dilutions (5-15% v/v dH_20) of bleach (6% w/v sodium hypochlorite) for 1-5 mins. After bleaching, fronds were transferred individually to sterile Petri dishes containing the same Modified Hoagland E+ growth medium described above. About 10 days later, Petri dishes were visually assessed for plant vitality and microorganism contamination (about 20% of fronds survived bleaching and had no signs of contamination), and a single, vital, non-contaminated frond was selected to initiate the sterile stock culture from which all subsequent plants were derived. Prior to the start of the study, the stock culture was switched from Modified Hoagland E+ growth medium to half-strength Schenk and Hildebrandt (S-H) growth medium (Sigma-Aldrich S6765), which I used for all subsequent parts of the study. The S-H medium was again supplemented with sucrose (6.7 g/L final concentration), yeast extract (0.067 g/L), and tryptone (0.34 g/L) to make potential microorganism contamination more easily detectable.

APPENDIX 3: METHOD FOR CREATING AXENIC STOCK CULTURES (CHAPTER 5)

From each of the 75 subsamples of *L. minor*, I derived an axenic, single-genotype stock culture following the protocol described in Hillman (1961, pp. 236-237). First, I selected 10-20 healthy-looking fronds from each subsample and rinsed

them with dH_2O. I then 'pre-cultured' the selected fronds for about 24 h in a Petri dish (one for each subsample) containing Modified Hoagland E+ (MHE+) liquid growth medium (recipe in Environment Canada 2007, pp. 12-14). The pre-culture period was intended to encourage microorganism growth and spore germination. After another dH_2O rinse, I submerged fronds individually in varying dilutions (5-15% v/v dH_20) of a commercially available bleach solution (6% w/v sodium hypochlorite) for 1-5 mins. I then transferred fronds to sterile Petri dishes (one frond per Petri dish this time) containing MHE+ growth medium, and placed the Petri dishes in plant growth chambers at 25˚C. About 10-15 days after bleaching, Petri dishes were visually assessed for plant vitality and microorganism contamination. For each of the 75 subsamples, I selected a single, surviving, non-contaminated frond to initiate a sterile stock culture from which experimental plants would be derived. Stock cultures were kept in Erlenmeyer flasks containing MHE+ growth medium and placed inside growth chambers at 25˚C with a 15:9 photoperiod. Prior to the start of the experiment, all stock cultures were transferred to half-strength Schenk and Hildebrandt (S-H) growth medium (Sigma-Aldrich S6765), which I supplemented with sucrose (final concentration 6.7 g/L), yeast extract (0.067 g/L), and tryptone (0.34 g/L) to make microorganism contamination more easily detectable.